



QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE.

EDITOR :

EDWIN S. GOODRICH, M.A., LL.D., F.R.S.,

INAGRE PROFESSOR OF ZOOLOGY AND COMPARATIVE ANATOMY IN THE UNIVERSITY OF OXFORD;

WITH THE CO-OPERATION OF

J. GRAHAM KERR, M.A., F.R.S.,

REGIUS PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF GLASGOW;

E. W. MACBRIDE, M.A., D.Sc., LL.D., F.R.S.,

PROFESSOR OF ZOOLOGY AT THE IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY;

G. P. BIDDER, Sc.D., SEC. L.S.;

E. J. ALLEN, D.Sc., F.R.S.,

DIRECTOR OF THE MARINE BIOLOGICAL LABORATORY, PLYMOUTH;

J. H. ASHWORTH, D.Sc., F.R.S.,

PROFESSOR OF NATURAL HISTORY IN THE UNIVERSITY OF EDINBURGH;

D. M. S. WATSON, M.Sc., F.R.S.,

DRELL PROFESSOR OF ZOOLOGY AND COMPARATIVE ANATOMY IN THE UNIVERSITY OF LONDON.

VOLUME 73. New Series.

WITH LITHOGRAPHIC PLATES AND TEXT-FIGURES



3644/36

OXFORD UNIVERSITY PRESS,

HUMPHREY MILFORD, LONDON, E.C.4.

1930

3644
IARI

PRINTED IN GREAT BRITAIN AT
THE UNIVERSITY PRESS, OXFORD
BY JOHN JOHNSON
PRINTER TO THE UNIVERSITY

CONTENTS

CONTENTS OF No. 289, N.S., July, 1929.

MEMOIRS:

PAGE

The Spermatogenesis of <i>Lepisma domestica</i> . By J. BRONTË GATENBY and R. N. MUKERJI. (With Plate 1 and 1 Text-figure).	1
Studies in the Origin of Yolk: III. Oogenesis of the Firefly, <i>Luciola gorhami</i> . By VISHWA NATH, M.Sc., Phil.D. (Cantab.), and DEV RAJ MEHTA, M.Sc. (With 16 Text-figures)	7
Studies on the Development of the Genitalia and the Genital Ducts in Insects. I. Female of Orthoptera and Dermaptera. By R. I. NEL, M.Sc. (Agric.). (With Plates 2-4 and 5 Text-figures)	25
On the Development of the Mid-Gut in the Larval Stages of <i>Vanessa urticae</i> (Lepidoptera). By H. HENSON, B.Sc. (With Plate 5 and 1 Text-figure)	87
<i>Physophaga sappheira</i> , n.g., n.sp. By MURIEL PERCY. (With Plate 6)	107
Lipin Secretion in the Elasmobranch Interrenal. By A. H. H. FRASER, M.B., Ch.B., B.Sc. (With Plates 7 and 8)	121
Mitochondrial Behaviour during the Life-cycle of a Sporozoon (<i>Monocystis</i>). By E. S. HORNING, D.Sc. (With Plate 9)	135
The Conus Arteriosus in Fishes. By C. W. PARSONS, B.A. (With 10 Text-figures)	145

CONTENTS OF No. 290, N.S., November, 1929.

MEMOIRS:

Nucleolar phenomena during Oogenesis in certain Tenthredinidae. By R. A. R. GRESSON, B.Sc. (With Plate 10 and 1 Text-figure)	177
Nucleolar behaviour in the Mitosis of Plant Cells. By PRISCILLA FREW and ROBERT H. BOWEN. (With Plate 11)	197
The Conjugation of a Triploid Chilodon. By MARY STUART MacDOUGALL. (With 16 Text-figures)	215
The Early Prophases of the First Oocyte Division as seen in Life, in <i>Obelia geniculata</i> . By G. H. FAULKNER, B.Sc. (Edin.). (With 22 Text-figures)	225
The Histology of the Alimentary Tract of the Plaice (<i>Pleuronectes platessa</i>). By BEN DAWES, A.R.C.S., D.I.C. (With 12 Text-figures)	243
The Gregurines of <i>Cucumaria</i> ; <i>Lithocystis minchinii</i> Woods and <i>Lithocystis cucumariae</i> n.sp. By HELEN PAXELL GOODRICH, M.A. (Oxon.), D.Sc. (Lond.). (With 4 Text-figures)	275
On the Development of the Skull of <i>Leptodeira hotamboia</i> . By GWENDOLEN T. BROCK, M.Sc., D.Phil. (Oxon.). (With Plates 12 and 13 and 6 Text-figures)	289
The formation and fate of the Operculum and Gill-chambers in the tadpole of <i>Rana temporaria</i> . By GWENDOLEN T. BROCK, M.Sc., D.Phil. (Oxon.). (With 16 Text-figures)	335
Yolk-formation in certain Tenthredinidae. By R. A. R. GRESSON, B.Sc. (With Plates 14 and 15 and 3 Text-figures)	345

CONTENTS OF No. 291, N.S., February, 1930.

DEMOIRS:

	PAGE
The Neuro muscular mechanism of the gill of <i>Pecten</i> . By S. B. SETNA, M.Sc. (With Plates 16-18 and 4 Text-figures) . . .	365
Metamerism in Enteropneusta. By Dr. C. J. VAN DER HORST. (With 3 Text-figures) . . .	393
The Cytology and Binary Fission of <i>Peranema</i> . By VIRGINIUS E. BROWN. (With Plates 19-21 and 1 Text-figure) . . .	403
Preliminary Studies on the Bacterial Cell-mass (Accessory Cell-mass) of <i>Calandra oryzae</i> (Linn.): The Rice Weevil. By K. MANSOUR, B.Sc., D.I.C., Ph.D. (With Plates 22, 23, and 4 Text-figures). . .	421
Variation in the Histological Condition of the Thyroid Glands of Sheep with regard to Season, Sex, Age, and Locality. By ESTHER LOWE, M.Sc. (With Plates 24 and 25) . . .	437
The Green Bodies of the Intestinal Wall of certain Chaetopteridae. By C. BERKELEY. (With Plate 26). . .	465
Studies on the shape of the Golgi Apparatus. II. Observations on the fresh Egg of the Indian Earthworm, <i>Pheretima posthuma</i> . By VISHWA NATH, M.Sc., Ph.D. (Cantab.). (With 17 Text-figures) . . .	477
The Development of the Amphibian Kidney. Part I. The Development of the Mesonephros of <i>Rana temporaria</i> . By PETER GRAY. (With Plates 27-31 and 8 Text-figures) . . .	507

CONTENTS OF No. 292, N.S., May, 1930.

DEMOIRS:

Le Cycle chromosomique d'une nouvelle Actinomyxidie: <i>Guyénotia sphaerulosa</i> N. gen.; n.sp. Par ANDRÉ NAVILLE. (Avec planches 32 à 34, et trois figures dans le Texte) . . .	547
Seasonal and sexual variation in the Thyroid Glands of Cats. By ESTHER LOWE, M.Sc. (With Plates 35-36) . . .	577
The formation of the Peritrophic Membrane in Insects, with special reference to the Larvae of Mosquitoes. By V. B. WIGGLESWORTH, M.A., M.D. (With 10 Text-figures) . . .	593
Certain phenomena of Tenthredinid Oogenesis as revealed mainly by Feulgen's Nuclear-Reaction. By R. A. R. GRESSON. (With Plate 37 and 4 Text-figures) . . .	617
The Cytological changes observable in irradiated Bean Root Tips. By RUTH E. P. PATTON, B.A., M.Sc. (Dubl.), and SYLVIA B. WIGODER, M.A., M.D. (Dubl.). (With Plates 38-40) . . .	633
On a new Hermaphrodite Syllid. By E. S. GOODRICH. (With 12 Text-figures) . . .	651
On the cranial characters of the South African Brevicipitid, <i>Phrynomerus bifasciatus</i> . By C. G. S. DE VILLIERS, M.A., Ph.D. (With 14 Text-figures) . . .	667
The early development of the Chondrocranium of the Lizard. By G. R. DE BEER, M.A., B.Sc., F.L.S., C.M.Z.S. (With 28 Text-figures) . . .	708

The Spermatogenesis of *Lepisma domestica*.

By

J. Brontë Gatenby

and

R. N. Mukerji.

From the Zoological School, Trinity College, Dublin.

With Plate 1 and 1 Text-figure.

INTRODUCTION

IN another paper¹ by the senior writer and Dr. Sylvia Wigoder on the post-nuclear granules in spermatogenesis it was pointed out that the interpretation of the spermatogenesis of *Lepisma* given by Robert Bowen (2, 3) was probably incorrect. Recently we have made a number of preparations of the *Lepisma* testis, and in the present paper we have been able to show that the spermateleosis of *Lepisma* is quite like that of other arthropods.

PREVIOUS WORK.

The only special investigation of *Lepisma* previously published is that of Harry H. Charlton, who confined himself almost entirely to the chromosome cycle. Charlton has, however, given some account of the cytoplasmic inclusions, and has paid some attention to the final steps in sperm formation. His interpretation of the structures in the lengthening sperm is given in Text-fig. 1, III. He describes an acrosome in front, as is usual, and a larger structure behind the nucleus, which he calls the 'middle-piece'. Charlton traces the 'middle-piece anlage' back into the early spermatid. Charlton does not quote the senior author's study on *Lepidoptera*, published five years before, and has been

¹ 'Proc. Roy. Soc.', B. v. 104, 1929.

unable to give a clear account of acrosome formation, or of the centrosome.

More recently Robert Bowen (2) has re-examined a number of Charlton's preparations, and has proposed a completely new interpretation of the parts of this spermatozoon, even suggesting that the *Lepisma* spermatozoon is unique, and of an 'atypical flagellate' nature.

In Text-fig. 1, I, the parts marked A, c, are according to Bowen the centrosome, and the structure PNB (Charlton's middle-piece) is the acrosome. The sperm-head is thus turned around the wrong way if Bowen's interpretation is correct. No other such sperm is known in the animal kingdom.

In Text-fig. 1, II, is a spermatid of a moth from the figures of Bowen, the lettering except for the acrosome (A) being ours. In such a sperm the acrosome is in contact, or placed near two other bodies, one, the centrosome, from which the flagellum takes its origin, the other the 'post-nuclear' granule of the senior writer.

TECHNIQUE.

The technique was that given in the senior writer's article in the 'Microtomist's Vade-Mecum'. In addition we used the neutral red intra vitam method, but we do not propose to describe the vacuolar system in the present paper. This will be done by one of us in a separate article.¹ The vacuolar system resembles that of moths, described by Hirschler and the senior writer.

PERSONAL INVESTIGATIONS.

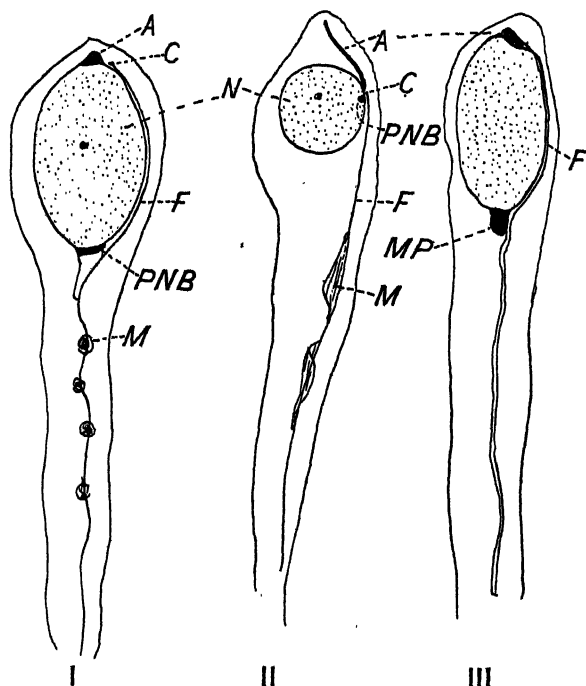
In fig. 6, Pl. 1, is the earliest stage depicted in our diagrams. The centrosome has divided into two (c^1 , c^2) and the flagellum is already well grown (F). Below the nucleus is the usual mitochondrial nebenkern (NK), and at GB are the two Golgi elements or discs. On the left side of the nucleus are two bodies, the larger spherical one being the chromatoid body, the smaller, elongate, often spindle-shaped one being the post-nuclear body, Charlton's 'middle-piece anlage'. The acrosome is yet unformed.

In the next stage, fig. 5, Pl. 1, the acroblasts (Golgi bodies)

¹ 'Jour. Roy. Micr. Soc.', March 1929.

are in situ (GA), while the post-nuclear body has visibly swollen. The nebenkern has become more chromophile, and the chromatoid body hovers near.

In fig. 3, Pl. 1, is a cell from the same nest, the nebenkern



TEXT-FIG. 1.

not being in sight. The post-nuclear body has become applied to one side of the nucleus (PNB), the other side showing a minute granule (really the centrosome and tip of the flagellum), and the Golgi body, one alone shown.

Now in the next stage, a good deal later, given in fig. 1, Pl. 1, the acrosome, A, has been formed and is in situ, the post-nuclear body is firmly fixed to the ovoid nucleus, and a peculiar sort of funnel (PNF) has appeared behind the post-nuclear granule. Running obliquely across this is the flagellum

(F) which passes forwards, behind the nucleus, and is found to pass up to the region of the acrosome. This is shown in fig. 7, Pl. 1. The peculiar post-nuclear funnel is shown in figs. 1, 2, 4, and 5, Pl. 1, and is indicated by Bowen in his fig. 107. In fig. 4, Pl. 1, the spermatid at about the same stage shows in front of the nucleus, two bodies, one very small and intensely staining—namely the centrosome, the other the acrosome; the tail with the peculiar mitochondrial blebs, passed back at m, as is also shown in fig. 7, Pl. 1.

In Bowen's figs. 110–18 further stages in spermateleosis are shown, and need not be repeated here. It should be remembered that the centrosome still lies near the head end of the nucleus, but behind the thread-like acrosome, while the structure marked acrosome (A) in Bowen's figures is the post-nuclear body, or if one wishes to use Charlton's nomenclature, the 'middle-piece'.

DISCUSSION.

The spermatogenesis of *Lepisma* follows the usual arthropod lines, except that the post-nuclear body is larger than is usual. Charlton's account of the ripening spermatozoon is quite correct except for the fact that he has lost the centrosome.

Text-fig. 1, I, II, and III, sufficiently explain the views of Charlton and the present writers. It is suggested that the term 'middle-piece' should not be used for the post-nuclear granules, because the latter is not the same thing as the middle-piece of the mammalian spermatozoon.

Bowen appears also to have misinterpreted Goldsmith's quite correct observations on coleopterous spermatogenesis. The matter is at present being investigated in this laboratory, but, as pointed out previously,¹ the middle-piece of Goldsmith's forms is not chromatinic, as Bowen has suggested, but is, as Charlton states, the exact homologue of the middle-piece of *Lepisma*—namely the post-nuclear granule.

TRINITY COLLEGE, DUBLIN,
October 1928.

¹ 'Proc. Roy. Soc.', B. v. 104, 1929.

LITERATURE.

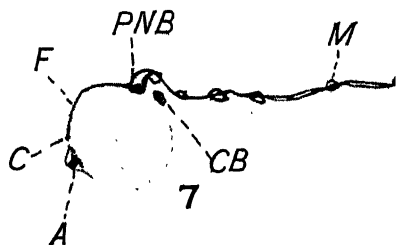
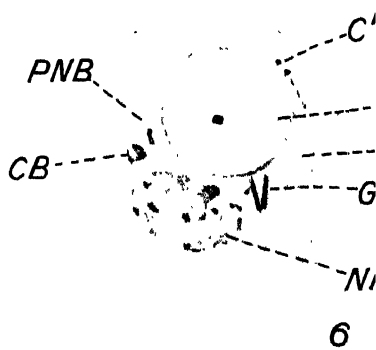
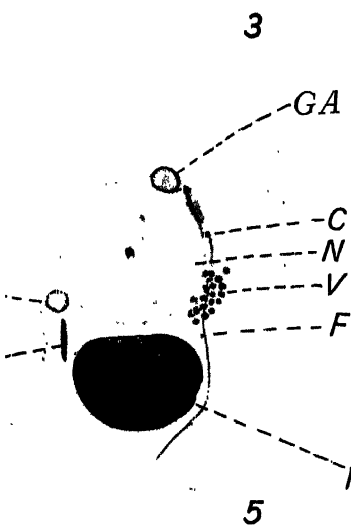
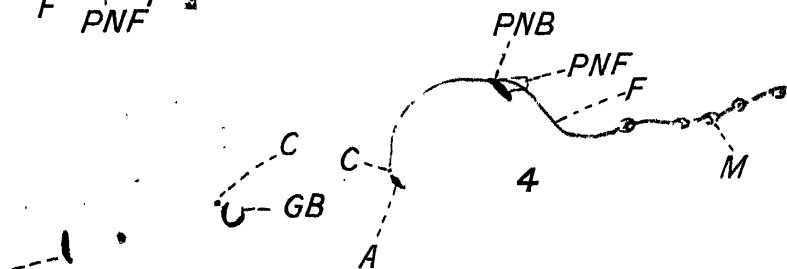
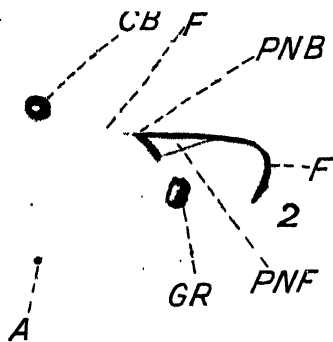
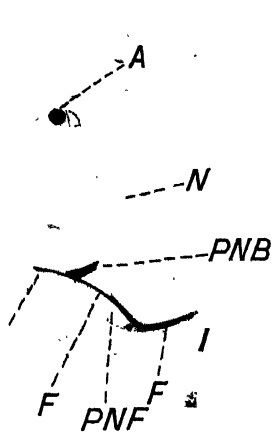
1. Charlton, Harry H. (1921).—"The spermatogenesis of *Lepisma domestica*", 'Journ. Morph.', vol. 35.
2. Bowen, Robert H. (1924).—"Studies on insect spermatogenesis", *ibid.*, vol. 39.
3. — (1925).—"Further notes on the acrosome of the animal sperm. The homologies of non-flagellate sperms", 'Anat. Record', vol. 31.
4. Gatenby, J. Brontë (1917).—"The cytoplasmic inclusions of the germ cells. Pt. I. Lepidoptera", 'Quart. Journ. Micr. Sci.'
5. Goldsmith, W. M. (1919).—"A comparative study of the chromosomes of the tiger beetles (*Cicindelidae*)", 'Journ. Morph.', vol. 32.
6. Hirschler, Jan (1927).—"Appareil de Golgi-vacuome au cours de la spermatogenèse chez *Macrothylacia rubi* L.", 'Compt. R. Soc. Biol.'

EXPLANATION OF PLATE 1.

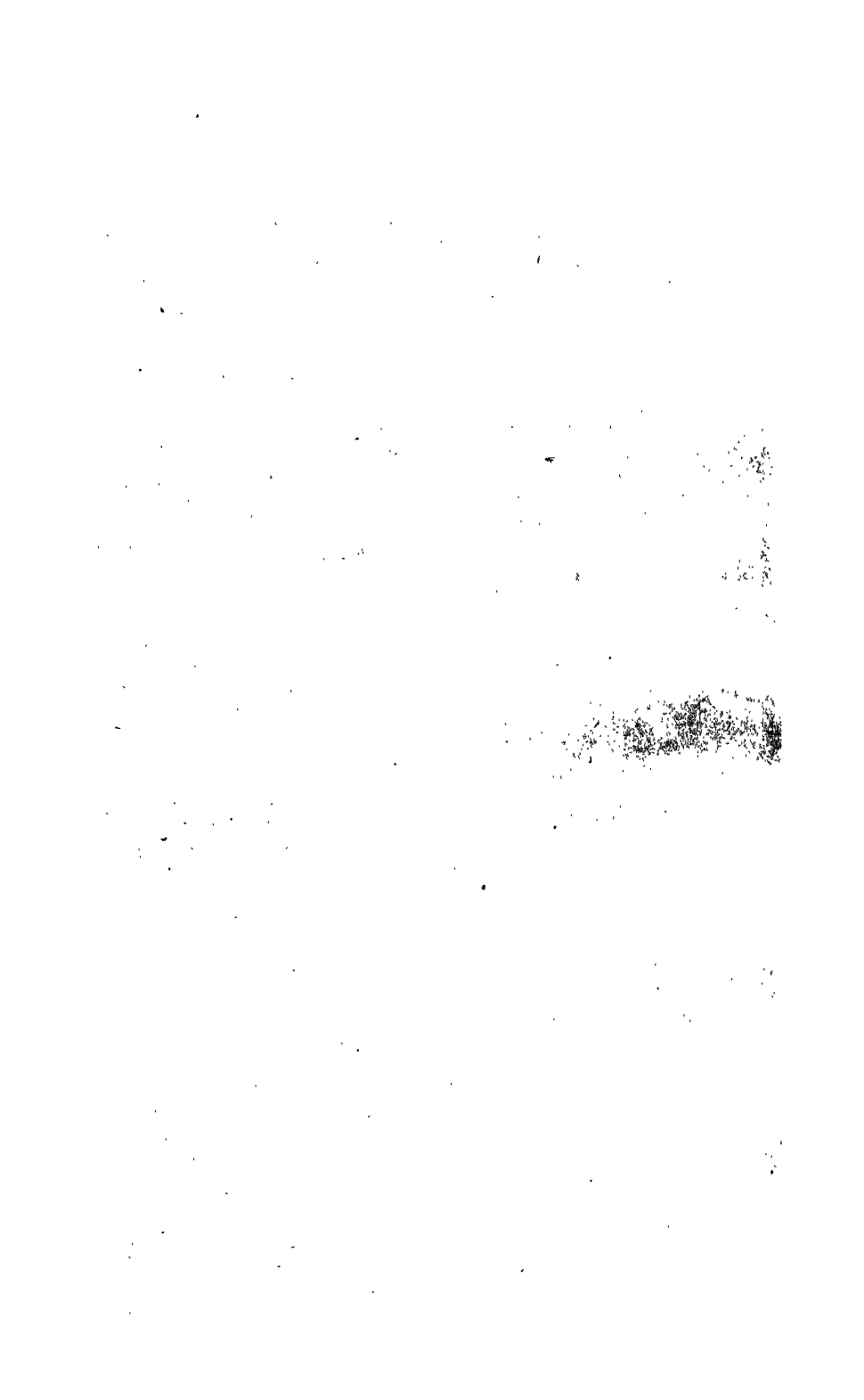
LETTERING.

A, acrosome; C, centrosome; CB, chromatoid body; F, flagellum; GB, Golgi body; GR, Golgi remnant; N, nucleus; NK, nebenkern; M, mitochondrial; PNB, post-nuclear body; PNF, post-nuclear funnel; V, vacuolar system. Technique: F.w.a., iron haematoxylin.

Figs. 1-7.—Spermatids of various ages.



el.



Studies in the Origin of Yolk.

III. Oogenesis of the Firefly, *Luciola gorhami*.

By

Vishwa Nath, M.Sc., Ph.D. (Cantab.),

and

Dev Raj Mehta, M.Sc.

Department of Zoology, Government College, University of the
Punjab, Lahore.

With 16 Text-figures.

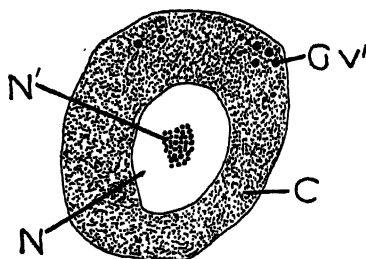
In the first two papers of this series¹ one of us has shown, by studying both fixed and fresh preparations stained with vital dyes, that the Golgi elements in the youngest oocytes of the spider and *Scolopendra* consist of vacuoles which, after treatment with various fixatives, do not show any coagulum inside their interior, indicating that their contents are watery and non-fatty. When the oocyte begins to enlarge, the majority of these vacuoles, by a process of growth and deposition inside their interior of colloids in the form of free fat not miscible with the general cytoplasm, give rise to fatty yolk-vacuoles, which henceforward show a distinct black coagulum inside their interior after treatment with osmic acid. By this process the refractive index of the Golgi vacuoles is considerably raised, and it becomes increasingly easier to study them in fresh preparations. In the case of the firefly, *Luciola gorhami*, however, which forms the subject of the present paper, we observed the remarkable thing that the Golgi vacuoles of the female primordial germ-cells contain free fat as proved by their blackening in 2 per cent. osmic acid in ten minutes. When the oocyte is

¹ 'Quart. Jour. Micr. Sci.', vol. 72, Parts II and III.

differentiated and begins to enlarge many of these vacuoles grow in size and give rise to fatty yolk-vacuoles. *Luciola*, therefore, is a very valuable material for the demonstration of the origin of the fatty yolk-vacuoles from the Golgi vacuoles, inasmuch as the latter are fatty from the very beginning.

The technique used is mentioned in the explanatory notes on

TEXT-FIG. 1.



Youngest oocyte. The surrounding follicle cells have not been shown.
Mann-Kopsch stained with acid fuchsin. $\times 1,120$.

EXPLANATION OF LETTERING.

A.Y., albuminous yolk; *C.*, cytoplasm; *F.C.*, follicle cells; *F.E.*, follicular epithelium; *G.V.*, Golgi vacuole; *G.V'*, Golgi vacuole looking solid on account of osmication; *M.*, mitochondria; *M'*, egg membrane; *N.*, nucleus; *N'*, nucleolus; *N''*, secondary nucleoli; *N.F.*, nucleus of the follicle cell; *O.*, oocyte.

the figures and also in the text. In addition to this routine technique one of us (V. N.) has used the vital dyes neutral red and Janus green B, as also the technique described below. While these dyes brought out most satisfactorily the Golgi vacuoles and the mitochondria in the primordial germ-cells, they totally failed to show these inclusions in the oocytes for the following reason. When the oocyte differentiates it is surrounded on all sides by a large number of follicle cells which do not allow a sufficient amount of light to pass through the follicle to enable one to see the inclusions in the oocyte as stained with neutral red or Janus green B. By the time most of the follicle cells are used up by the oocyte for nourishment and the remaining ones form a thin follicular epithelium round it, albuminous yolk

OOGENESIS OF LUCIOLA

TEXT-FIGS. 2 AND 3.

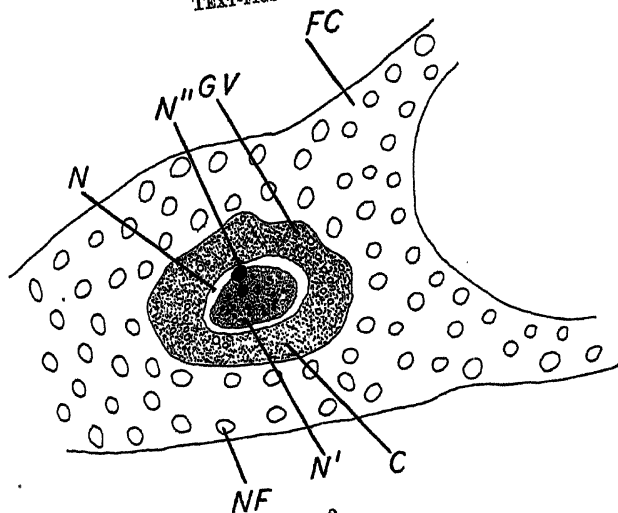


FIG. 2.

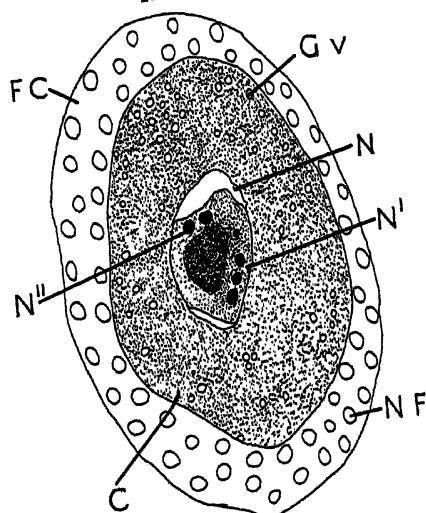
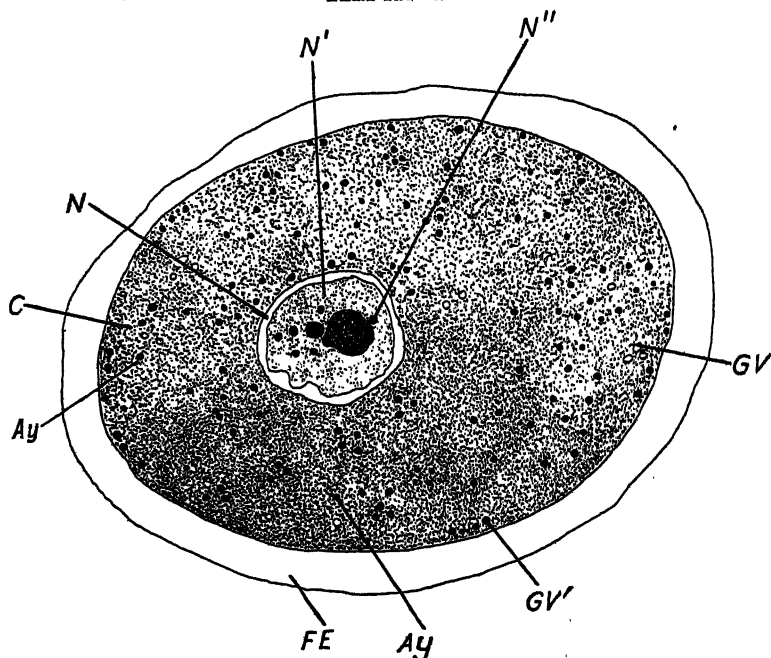


FIG. 3.

A little more advanced oocytes. Only the nuclei of the follicle cells have been shown as the cell boundaries did not come up well. The Golgi vacuoles of the follicle cells have not been drawn. Mann-Kopsch stained. Both are magnified 520 times.

appears in the oocyte and makes it opaque. Another technique, therefore, had to be used. The ovary is kept in 2 per cent. osmic for about ten minutes and is then studied in a drop of water under the microscope. Both the Golgi vacuoles and the fatty

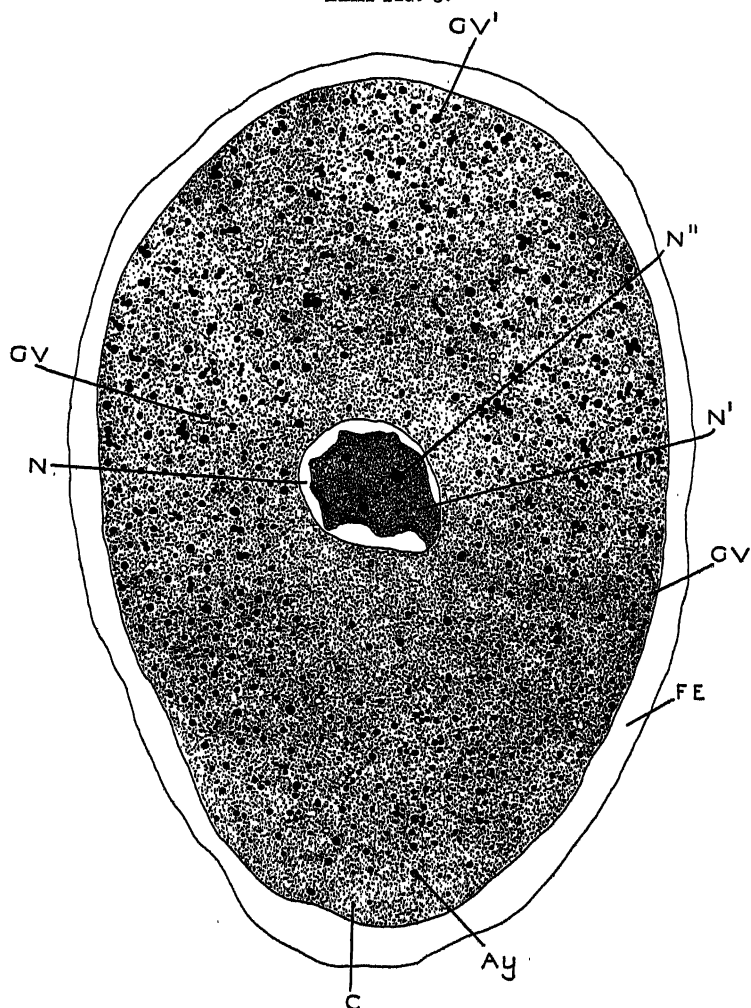
TEXT-FIG. 4.



Text-figs. 4 and 5. Still more advanced oocytes. The follicle cells are now arranged in a single-layered epithelium whose details have been omitted. Albuminous yolk is beginning to appear. Mann-Kopsch stained. Text-fig. 4 magnified 420 times, and Text-fig. 5 magnified 360 times.

yolk-vacuoles are blackened by the OsO_4 , but not so much as to make them look solid as they do after prolonged treatment with this acid. During this short period the vacuoles become slightly black, with the result that each vacuole shows a black chromophilic rim and a central clear substance. This method proved highly satisfactory, and brought out the vacuoles in all stages of oogenesis. As was expected it failed to show the mitochondria. This technique cannot be strictly called 'vital', but

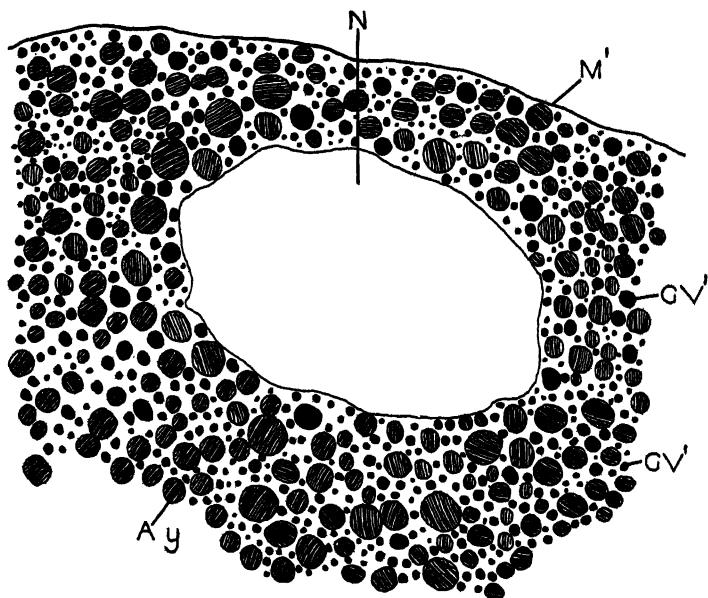
TEXT-FIG. 5.



it comes very near it as the amount of the coagulation of the protoplasm is very small, and there is hardly any possibility of an artifact being introduced. Experience has shown us that osmic acid used for a few minutes is the best fixative known. All the diagrams have been drawn by Vishwa Nath.

It is a great pleasure to us to thank Colonel S. R. Christophers, F.R.S., Director of the Central Research Institute, Kasauli, for giving us all facilities for this work in his laboratory in the summer of 1926, and to the authorities of the Indian Museum for supplying us with the necessary literature.

TEXT-FIG. 6.



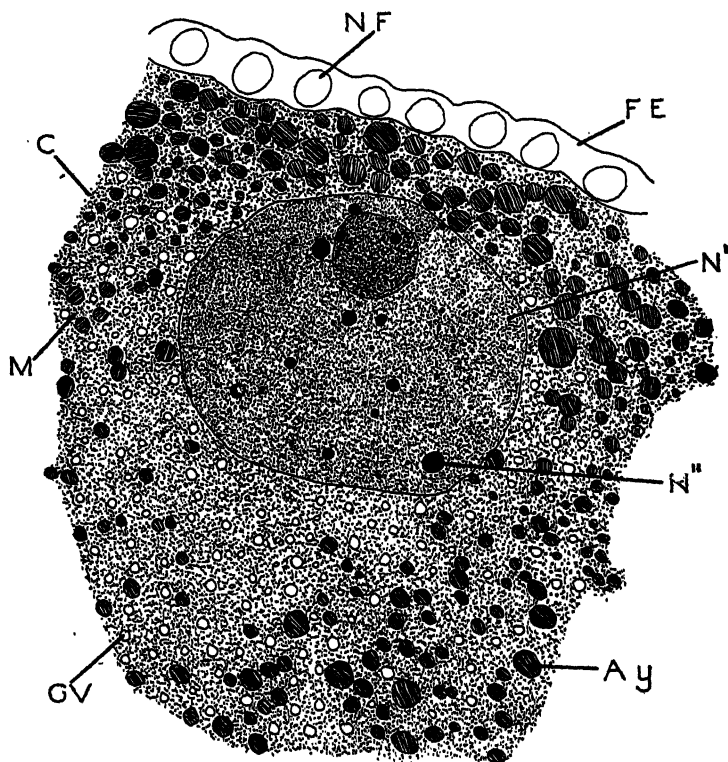
A portion of the most highly advanced ovarian oocyte. The follicular epithelium has disappeared and the egg membrane has appeared. Champy-Kull unstained. $\times 500$.

OBSERVATIONS.

The ovary is kept in 2 per cent. osmic acid for about ten minutes, mounted in a drop of water on a slide, covered with a cover-slip, and studied under the $\frac{1}{2}$ objective. Text-figs. 12-16 are drawn from such a material. Text-fig. 16 represents the greater portion of the sac containing the primordial germ-cells. The nuclei of these germ-cells appear distinctly, but the cell boundaries cannot be made out. In the spaces between the

nuclei one can see distinct vacuoles with a rim gone black due to osmication and a clear central substance. These are the Golgi vacuoles. If, however, the ovary is kept in 2 per cent. osmic acid for more than half an hour, these vacuoles are blackened

TEXT-FIG. 7.

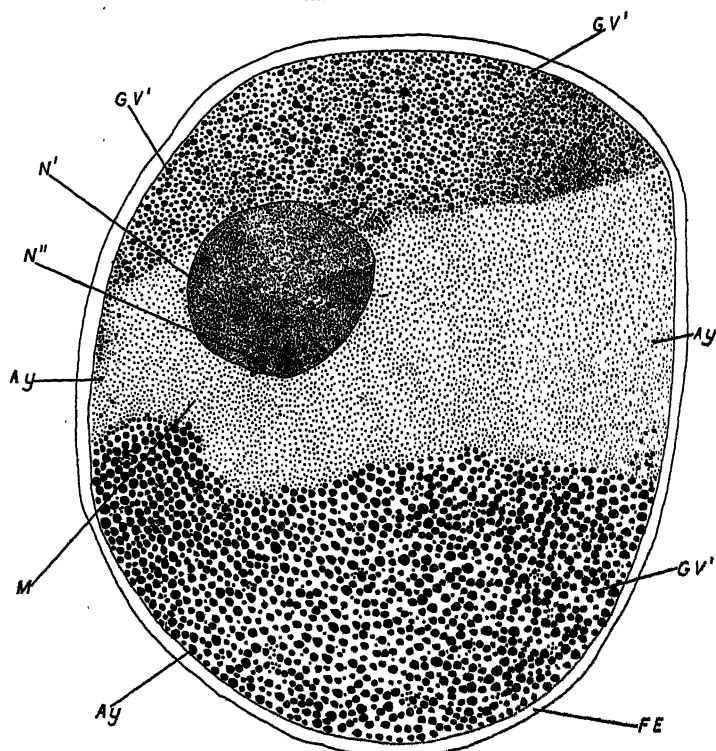


A portion of an oocyte younger than that represented in Text-fig. 6. Champy-Kull stained with acid fuchsin. $\times 500$.

considerably and then appear solid. Such quick blackening of these vacuoles in osmic acid unmistakably shows that their contents are fatty. The number of these vacuoles is about three or four in each cell, as can be determined more accurately by the study of fixed preparations, and they are not closely aggregated in each cell. In chrome-osmium and Mann-Kopsch preparations

they appear solid and black, but they can be easily decolorized with xylol or turpentine, after which they appear as clear vacuoles. With Janus green B or neutral red, in which the ovary is kept for about fifteen minutes, they appear as clear

TEXT-FIG. 8.



A centrifuged oocyte. Mann-Kopsch stained with acid fuchsin. $\times 160$.

vacuoles, and the mitochondria are stained green or red according to the vital dye used but they are much more distinct after treatment with Janus green B. For reasons already explained these vital dyes fail to show either the mitochondria or the Golgi vacuoles in the oocytes. When the oocyte is differentiated from the follicle cells 2 per cent. osmic is the only reagent which shows the Golgi vacuoles in fresh preparations.

In Text-fig. 12 the oocyte has differentiated from the surrounding follicle cells. The Golgi vacuoles are found at the periphery of the cytoplasm of the oocyte. Why these vacuoles are present only at the periphery we are unable to explain. We are, however, certain that this peripheral distribution is a constant feature of the oocytes of this age. In Text-fig. 13 the oocyte has grown in size, and the Golgi vacuoles have increased and are uniformly distributed throughout the cytoplasm. The nucleoli have become prominent and are shown at *N''*. They appear solid and colourless or very slightly brownish. Although the nuclear membrane cannot be made out the size of the nucleus can be more or less determined by the clear space round the group of nucleoli. Text-fig. 14 represents an older oocyte. The albuminous yolk has begun to be deposited and appears as colourless or very slightly brownish, solid, and homogeneous discs. The Golgi vacuoles have increased in number and are distributed uniformly throughout the cytoplasm. They show a very distinct black rim and a clear interior, and are thus very easily distinguished from the albuminous yolk. The nucleoli are increasing in number and the nucleus is represented by a clear space round them. After the stage represented in Text-fig. 14 the oocyte is quickly packed with albuminous yolk, which makes it very opaque and unfit for study in whole mounts. When such an oocyte is treated with 2 per cent. osmic for a short time, it becomes dark and unfit for study unless sections are cut. If, however, it is ruptured by a needle its contents which flow out can be studied with remarkable ease. Text-fig. 15 represents a fragment of an osmicated and highly developed oocyte which has been ruptured with a needle. The albuminous yolk appears as colourless, solid, and homogeneous discs, while the Golgi vacuoles of different sizes are blackened with OsO_4 and occupy the spaces between the yolk-discs.

Text-figs. 1-5 are stained Mann-Kopsch preparations. Text-fig. 1 represents the youngest oocyte that we have been able to obtain. At *G.V'* are the Golgi vacuoles that look solid on account of osmication. In Text-figs. 2 and 3 the vacuoles have increased in number and have been decolorized by xylol. In Text-fig. 4,

TEXT-FIGS. 9, 10, AND 11.

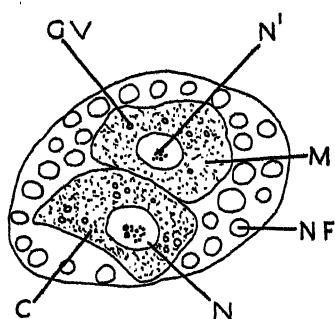


FIG. 9.

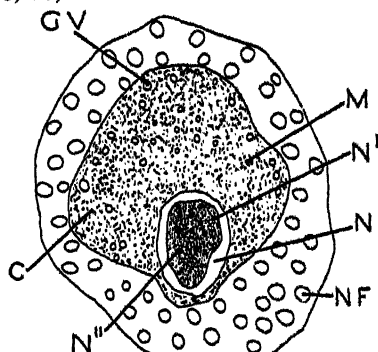


FIG. 10.

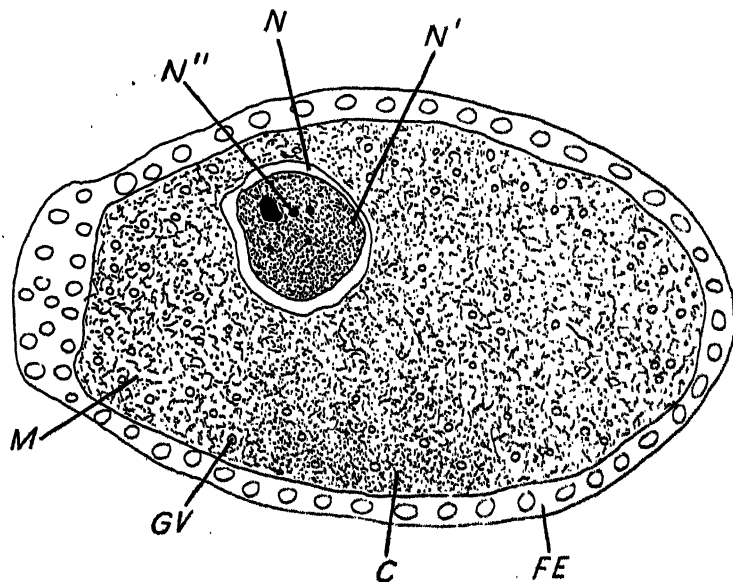


FIG. 11.

Young oocytes showing Golgi vacuoles and mitochondria. Champy-Kull and iron haematoxylin. Text-figs. 9 and 10 $\times 500$, 11 $\times 420$.

which is a more advanced oocyte than that represented in Text-fig. 8, most of the Golgi elements look solid on account of osmication, but some of them shown at G.V. are decolorized by xylol and appear as clear vacuoles. In Text-fig. 5, which is a still more

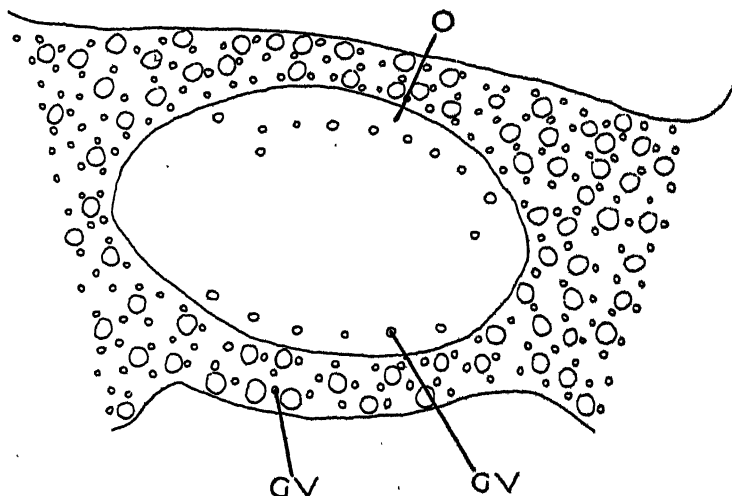
advanced oocyte, many Golgi elements have swollen up. Most of them look solid and black on account of osmication. Nevertheless, xylol has decolorized some, e.g. at *G.V.* Experience with a large number of different eggs shows that xylol or turpentine does not decolorize all the osmicated fatty bodies at the same rate. Probably this is connected with the amount of free fat present inside these vacuoles, and the degree of coagulation that it undergoes in osmic acid. In all these preparations (Text-figs. 1-5) the mitochondria cannot be seen because it is very difficult to stain them after keeping the ovary in 2 per cent. osmic acid for from fourteen days to three weeks. Text-figs. 9-11 are chrome-osmium preparations stained with iron-alum haematoxylin. In all these preparations the osmicated Golgi elements have been decolorized by xylol and appear as clear vacuoles. Naturally enough, xylol will decolorize much more rapidly the osmicated Golgi vacuoles of oocytes fixed in chrome-osmium than the vacuoles of those oocytes fixed in Mann-Kopsch. In the former technique the tissue is exposed to osmic acid for about twenty-four hours, whereas in the latter the tissue is kept in the acid for from fourteen days to three weeks. In these preparations the mitochondria appear as small granules very sharply stained with haematoxylin, whereas the general cytoplasm is of a much lighter colour. Text-fig. 7 represents a portion of an advanced oocyte fixed according to the Champy-Kull method and stained with acid fuchsin. The osmicated Golgi elements have been decolorized by xylol and appear as clear vacuoles of different sizes. The mitochondria are strongly fuchsinophil and are uniformly distributed throughout the cytoplasm. Text-fig. 6 represents a portion of a most highly developed unstained ovarian oocyte prepared according to the Champy-Kull technique. The Golgi vacuoles are osmicated and are not decolorized by xylol as the slide is passed quickly through it, whereas the albuminous yolk-discs are yellowish in colour.

In the above account we have deliberately avoided the words fatty yolk for obvious reasons. In the eggs of spiders and *Scelopendra* the Golgi vacuoles of the youngest oocyte contain a watery non-fatty substance. By a process of growth and

deposition inside them of free fat they give rise to the fatty yolk-vacuoles. As soon as free fat appears in the Golgi vacuoles they are called fatty yolk-vacuoles. But in the case of *Luciola*, free fat is present in the Golgi vacuoles of even the undifferentiated germ-cells, so that it is impossible to determine at

TEXT-FIGS. 12-16. These are drawn from fresh material kept in 2 per cent. osmic for about ten minutes.

TEXT-FIG. 12.



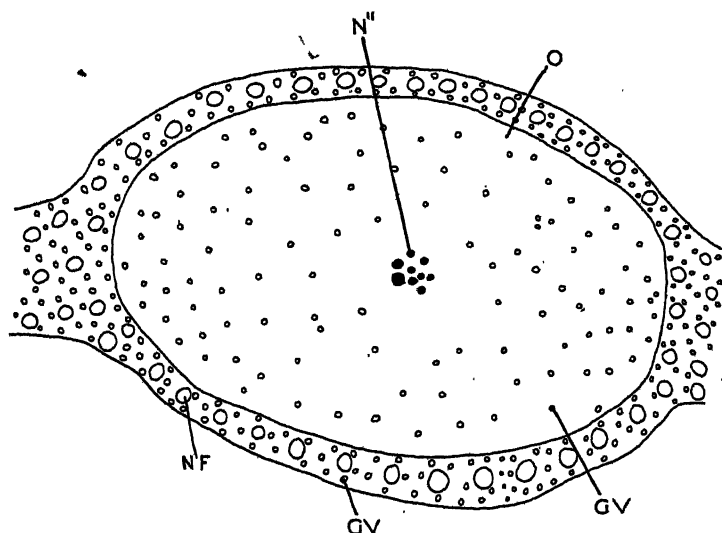
A young oocyte showing Golgi vacuoles at the periphery. The follicle cells also show these vacuoles. $\times 330$.

what stage of swelling a Golgi vacuole may be called a fatty yolk-vacuole. To avoid this confusion we have called these vacuoles the Golgi vacuoles irrespective of their size. It is highly probable that the big vacuoles will be used as nourishment during embryogeny; but it is impossible to be certain about this unless we study the segmentation of the fertilized egg.

The behaviour of the oocyte nucleolus is remarkable. In the primordial germ-cells the nucleolus is a small basophil body; but as soon as the oocyte is differentiated from the follicle cells it breaks up into a number of smaller basophil pieces which lie closely aggregated (Text-figs. 1 and 9). Indeed, this is the first

sign of the differentiation of the future oocyte. Gradually these basophil nucleolar pieces move apart, and they are then seen to be embedded in an acidophil ground-substance of plastin (Text-figs. 2 and 10). The whole nucleolus, therefore, is amphophil at this stage with an acidophil ground-substance embedded in which are round basophil bodies of different sizes.

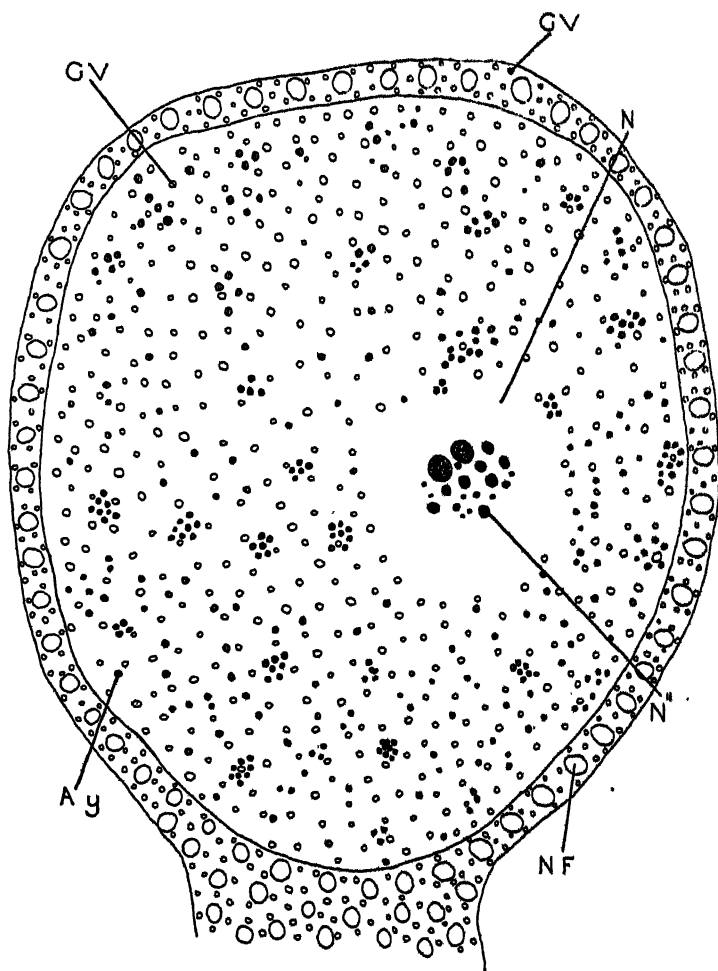
TEXT-FIG. 13.



A more advanced oocyte showing the increase and the uniform distribution of the Golgi vacuoles. Nucleoli are also shown with a clear space round them which represents the nucleus. $\times 290$.

Some of these bigger basophil bodies again become amphophil in exactly the same way as the original nucleolus did (Text-figs. 3, 4, and 7). The plastin ground-substance of the nucleolus goes on growing (Text-figs. 2, 3, 4, 5, 10, and 11) till it completely occupies the whole space within the nuclear membrane (Text-fig. 7). At the stage represented in Text-fig. 4 the basophil round nucleolar bodies begin to migrate into the cytoplasm of the oocyte and directly give rise to the albuminous yolk. In Text-fig. 5 this process has proceeded a little farther, and in Text-fig. 7 it has reached an advanced stage. There seems to be little

TEXT-FIG. 14.

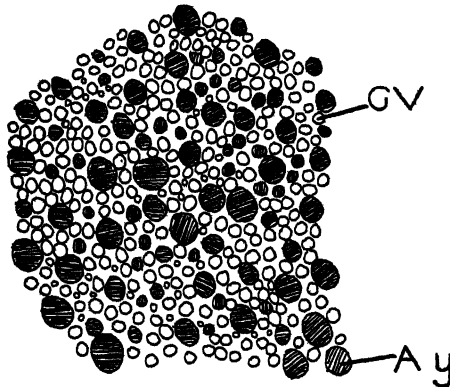


A still more advanced oocyte showing albuminous yolk and the increase in the number of the Golgi vacuoles. $\times 250$.

doubt that the nucleolar extrusions pierce the nuclear membrane as whole bodies. In the cytoplasm the nucleolar extrusions do not divide further but simply grow in size. The amount of albuminous yolk in a fully developed oocyte is very large (Text-fig. 6), but the nucleolar bodies are also numerous

(Text-figs. 5 and 7) and there can be no doubt that they give rise to this type of yolk. Besides the nucleolar activity synchronizes with the appearance of yolk, and the histochemical reactions of both types of bodies are exactly similar. Further, it is noteworthy that the migration of the nucleoli into the cytoplasm does not stop with the appearance of albuminous yolk as it does in *Lithobus* (Nath, 1924) and *Buthus* and

TEXT-FIG. 15.



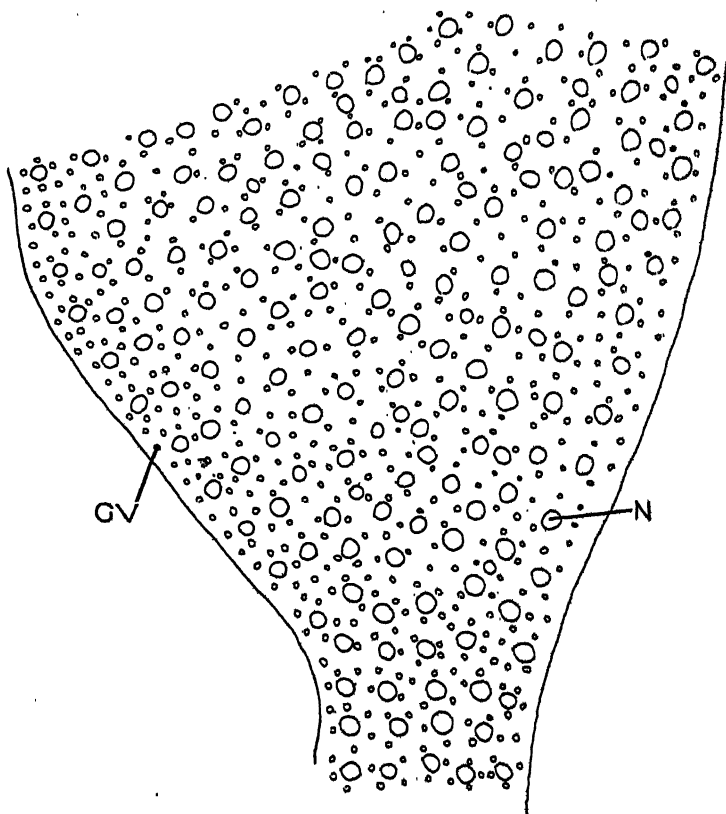
A fragment of a highly advanced oocyte showing albuminous yolk and Golgi vacuoles.

Euscorpius (Nath, 1925), but continues till the oocyte is fully grown.

We have sometimes observed a phenomenon in fixed preparations, namely, that at about the stage represented in Text-fig. 14 the albuminous yolk is arranged mostly at the periphery of the oocyte. At first we thought that this migration towards the periphery is real, and is undertaken with a view to derive nourishment for growth from the follicle cells. But an extensive study of fresh oocytes has convinced one of us that this peripheral position of the yolk-discs is really an artifact. How exactly this artifact comes about we cannot definitely explain. Possibly when the ovaries are placed in the fixatives most of the yolk-discs are carried towards the peripheral cytoplasm along with the diffusion currents that might be caused by the fixatives.

Experiments with the centrifuge gave us very satisfactory results. Text-fig. 8 represents an advanced centrifuged oocyte fixed with Mann-Kopsch and stained with acid fuchsin. At the

TEXT-FIG. 16.



Greater part of the sac containing the primordial germ-cells. Nuclei and Golgi vacuoles are shown but the cell boundaries cannot be seen.

lower pole are the heavy albuminous yolk-discs that are strongly fuchsinophil and at the opposite pole are the osmicated Golgi vacuoles of all sizes. The central area consists of granular mitochondria that did not stain very strongly, and also contains the nucleus which is always slightly pushed into the pole of the

Golgi vacuoles. It will be observed that a few osmicated Golgi vacuoles are present in the pole of the albuminous yolk, but this is due to mechanical causes inasmuch as they are caught between the big yolk-discs.

DISCUSSION.

With regard to the origin of fatty yolk-vacuoles from the Golgi vacuoles we do not wish to say more except emphasize the very important fact that in *Luciola* the Golgi vacuoles of even the undifferentiated germ-cells contain a colloid in the form of free fat inside them, whereas the Golgi vacuoles of the youngest oocytes of the spider and *Scolopendra* contain a watery non-fatty substance. In the latter cases these vacuoles by a process of growth and deposition of free fat inside them give rise to fatty yolk-vacuoles. In *Luciola*, however, the Golgi vacuoles simply grow in size and give rise to the fatty yolk-vacuoles. It is likely that in this process of growth more and more free fat is deposited in their interior.

The process of the origin of albuminous yolk from nucleolar extrusions is reminiscent of what has been described in the cockroach by Hogben (1920) and in *Saccocirrus* by Gatenby (1922). Nucleolar extrusions preceding the appearance of albuminous yolk have been described by one of us in *Lithobius* (1924) and *Buthus* and *Euscorpius* (1925), and by various other writers both in vertebrates and invertebrates. But in *Luciola* it is noteworthy that the process of nucleolar budding lasts practically throughout oogenesis, and the process of the growth of nucleolar extrusions into the albuminous yolk-spheres can be studied with diagrammatic clearness.

SUMMARY.

1. Both fresh and fixed eggs have been studied.
2. A remarkable phenomenon has been discovered in *Luciola*: that the Golgi elements which are in the form of vacuoles contain free fat even in the undifferentiated germ-cells.
3. The Golgi vacuoles containing free fat swell up in the oocytes and give rise to fatty yolk-vacuoles.

4. In the youngest oocyte the nucleolus is amphophil, consisting of deeply basophil round bodies, the nucleoli, embedded in an acidophil ground-substance.

5. The plastin ground-substance of the nucleolus grows and entirely fills up the whole space within the nuclear membrane.

6. The nucleoli multiply rapidly, migrate into the cytoplasm, and directly give rise to albuminous yolk.

7. Nucleolar budding lasts throughout oogenesis.

8. The mitochondria are granular and remain so throughout oogenesis.

BIBLIOGRAPHY.

1. Gatenby, J. Brontë (1922).—‘*Quart. Journ. Micr. Sci.*’, vol. 66.
2. Hogben, L. (1920).—‘*Proc. Roy. Soc. Lond.*’, Series B, vol. 91.
3. Nath, V. (1924).—‘*Proc. Phil. Soc. Camb. Biol. Sciences*’, vol. i.
4. — (1925).—‘*Proc. Roy. Soc. Lond.*’, Series B, vol. 98.

Studies on the Development of the Genitalia and the Genital Ducts in Insects. I. Female of Orthoptera and Dermaptera.

By

R. I. Nel, M.Sc. (Agric.),

Willem Hiddingh Scholar of the University of Stellenbosch,
South Africa.

From the Department of Entomology, Imperial College of Science and
Technology, London, S.W. 7.

With Plates 2-4 and 5 Text-figures.

CONTENTS.

	PAGE
I. INTRODUCTION	26
II. HISTORICAL	27
(a) Genitalia	27
(b) Genital Ducts	29
III. MATERIAL AND METHODS	32
IV. OBSERVATIONS ON LOCUSTANA AND COLEMANIA	33
(a) Adult Organs	33
(b) Development of the Genitalia	35
(c) Development of the Genital Ducts and Accessory Organs	37
V. OBSERVATIONS ON BLATTELLA	41
(a) Adult Organs	41
(b) Development of the Genitalia	43
(c) Development of the Genital Ducts and Accessory Organs	45
VI. OBSERVATIONS ON FORFICULA	46
(a) Adult Organs	46
(b) Development of the Genital Cavity, Genital Ducts, and Spermatheca	48
VII. DISCUSSION	49
A. Genitalia	49
B. Genital Ducts and Accessory Organs	51
(a) The Common Oviduct	52
(b) The Primitive Position of the Gonopore in Orthoptera and Dermaptera	55

	PAGE
(c) The Gonopore and Common Oviduct in Insects in	
General	58
(d) The Accessory Genital Organs	62
(e) The Spermatheca	67
(f) Concluding Remarks	71
VIII. SUMMARY AND CONCLUSIONS	72
IX. ACKNOWLEDGEMENTS	73
APPENDIX	74
BIBLIOGRAPHY	75
EXPLANATION OF PLATES	81

I. INTRODUCTION.

THE genital ducts and their associated accessory organs in insects have not yet been given the attention their morphological and phylogenetic interest merits. One finds in the extensive literature on the reproductive system that these organs have to a large extent received only incidental notice, the gonads and the external genitalia having claimed all the attention. Consequently it is not surprising that many details of their morphology are obscure on matters of dispute—as indeed a somewhat confused terminology testifies—and that the more interesting field of their phylogeny has scarcely been touched upon. While hardly any one doubts the monophyletic origin of insects, a hypothetical ancestral condition of the genital ducts and associated organs from which the diverse conditions existing in present-day forms can be derived still remains to be deduced. In the female this subject seems to entail a consideration of the following main problems: (1) significance of the mesodermal oviducts with respect to the gonopore or gonopores; (2) origin (azygous or paired, segmental position, &c.) and evolution of the ectodermal common oviduct; (3) origin and evolution of the accessory genital organs such as the spermatheca, accessory glands, &c.; and (4) the relations of the internal genital ducts to one another and to the external genitalia. The elucidation of these problems appears to me to depend largely on the evidence derivable from ontogeny. Such evidence is as yet very scanty as only a few detailed studies have been made on the

development of the organs in question. And it is noteworthy that these are devoted almost exclusively to the higher orders of insects, little or no work having been carried out on the phylogenetically ancient Orthoptera, Dermaptera, Isoptera, Plecoptera, Ephemerida, and Thysanura, which may reasonably be expected to yield valuable evidence. It has therefore seemed worth while to undertake ontogenetic studies on the genital duct system of some of these neglected insects. The present paper embodies my observations on the female of the Orthoptera *Locustana pardalina* Walk., *Colemania sphenarioides* Bolivar (Locustidae, olim Acridiidae), *Blattella germanica* L. (Blattidae), and the Dermapteron *Forficula auricularia* L. (Forficulidae). The genitalia are also considered because of their intimate relation with certain of the genital invaginations and because a reinterpretation of their homology is indicated. A second paper to follow shortly will be devoted to the male genitalia and genital duct system of the same insects. A study of Thysanura (Machilidae and Lepismidae) has been commenced and it is hoped to deal with their genital organs in the near future.

II. HISTORICAL.

(a) Genitalia.

The earliest morphological interpretation of the female genitalia was that of Lacaze-Duthiers (1849-53), who considered the gonapophyses to be modified dorsal and ventral pieces of the ninth abdominal segment. First opposed by Weismann (1866), this view yielded place to the hypothesis that the gonapophyses represented modified ventral abdominal appendages homodynamous with the legs (Ganin, 1869; Packard, 1871; Uljanin, 1872; Kraepelin, 1873; Dewitz, 1875; Huxley, 1877; Cholodokowsky, 1891). Dewitz found that in *Locusta* (*Phasgonura*) the valves of the ovipositor could be traced in the first instance to a pair of 'buds' on the eighth and ninth abdominal segments in the late embryo, the pair on the ninth giving rise in the nymph to a pair of outgrowths on their inner

margins. However, the ontogenetic evidence was not satisfactory until Wheeler (1893) showed in another Orthopteron, *Xiphidium*, that the 'buds' on the eighth and the outer 'buds' on the ninth originated from the early embryonic limb rudiments which were known to be homodynamous with the leg rudiments on the thorax. Nevertheless Grassi (1888), Haase (1889), Peytoureau (1895), on comparative anatomical grounds, and Heymons (1895-9), on ontogenetic evidence, considered that the gonapophyses represented specially developed structures which could not be referred to former limbs. Heymons found that the embryonic limb rudiments give rise to the styli. Verhoeff, in a series of papers (1895-8), adduced additional evidence in support of the appendicular origin of the gonapophyses, criticized Heymons's interpretations, and pointed out that the abdominal styli are serially homologous with the styli present on the legs of Machilidae and that these styli represent secondary coxal appendages. Building on the appendicular hypothesis, Verhoeff (1902) developed an interpretation of the genital segments and the gonapophyses which has been accepted by the majority of entomologists with but slight modifications, e.g. Börner (1904), Escherich (1905), Crampton (1917-25), Walker (1918, 1922), Bekker (1925), and others. According to Verhoeff the lateral divisions of the eighth and ninth sternal plates in Machilidae (*Thysanura*) represent flattened leg coxae, the gonocoxites, whereas the ovipositor lobes borne mesally by these structures, a pair each by the eighth and ninth gonocoxites, represent modified distal leg segments or telopodites (endopodites). In pterygote insects Verhoeff considers the anterior ovipositor valves of the eighth segment and the inner valves of the ninth to be serially homologous structures and to correspond to the telopodites of *Thysanura*; the lateral ovipositor valves of the ninth segment he homologizes as gonocoxites. While a few authors still subscribe to Heymons's views, e.g. van der Weele (1906), Chopard (1920), Hirschler (1924-7), Ludwig (1926), they do not produce evidence to challenge Verhoeff's interpretations. Nor does Tillyard (1926), who partially inclines to Heymons in that he regards the anterior

and inner ovipositor valves to be specially developed structures while believing the lateral valves to represent gonocoxites.

(b) Genital Ducts.

Previous to the work of Nusbaum (1882, 1884), observations on the development of the genital ducts and their accessory organs were few and imperfect, for, apart from the early work of Herold (1915) and Suckow (1928), these organs were but summarily dealt with in developmental studies, e.g. Weismann (1864, 1866), Bessels (1867), Ganin (1869), and others. According to Nusbaum, in *Lipeurus* and *Goniocotes* (Mallophaga) and in *Blatta* (Orthoptera), two cords stretching posteriorly from the young gonads give rise to the paired oviducts, the rest of the genital duct system (uterus, vagina, receptaculum seminis, and accessory glands) arising from the hypoderm. And as this worker found these hypodermal organs to originate from paired rudiments, he concluded that their unpaired condition in the adult is secondary. Almost at the same time Palmen (1884) showed that a common oviduct of hypodermal derivation is absent in *Ephemera*, the paired mesodermal oviducts opening separately in the adult in the intersegmental membrane between sterna 7 and 8. Further comparative anatomical considerations led Palmen to conclude that the paired openings of the oviducts behind the seventh sternum represents a primitive condition from which, by the addition of an unpaired terminal ectodermal portion, the efferent system of some other insects was derived. Jackson (1890) in *Vanessa* (Lepidoptera) found the rudiments of the paired oviducts to terminate in the seventh segment in the larva, thus representing an 'Ephemerid' stage, and the common oviduct and the accessory organs to develop later from the hypoderm. According to this worker the common oviduct is of unpaired origin, being derived from a ventral furrow on the eighth sternum, which becomes tubular anteriorly, and a similar furrow on the ninth in continuation with it, the lips of the furrow fusing ventrally and becoming tubular. Bilobed invaginations from the eighth and ninth sterna, from the roof of the furrows, give

rise the one to the bursa copulatrix and receptaculum seminis, the other to the sebaceous glands. Verson and Bisson (1896) in *Bombyx* (Lepidoptera), while differing from Jackson in describing the details of development, confirm his main conclusions. In Orthoptera, Heymons (1891) in *Phyllodromia* and Wheeler (1898) in *Xiphidium* showed conclusively that the paired oviducts are of mesodermal origin and that they terminate on the posterior margin of the seventh sternum. Wheeler further showed that the posterior ends of the oviducts are dilated into ampullae with a definite lumen, a persisting remnant of the coelomic cavity. Heymons's later and more extensive studies in Orthoptera (1895 a) confirmed these facts in an indubitable manner. Heymons and Wheeler further describe an invagination originating between the seventh and eighth sterna in the embryo, the 'vaginal' invagination, and challenge Nusbaum's idea of a paired origin of this structure. The first comprehensive treatment of the genital ducts and their accessory organs is due to Berlese (1909), whose discussion, although somewhat speculative and based largely on comparative anatomical data, often inaccurate, is none the less valuable. As regards the gonopore, Berlese states that it is not possible to deduce a common type for insects. He recognizes, however, a tendency for the gonopore to be more posteriorly placed in the higher insects (on the ninth sternite in Coleoptera, Heteroptera, and Diptera), and believes that its location on the eighth sternum in Orthoptera tends towards a primitive condition exhibited in the embryo of Coleoptera, where an invagination exists between sterna 7 and 8. With Brüel (1897) the paired oviducts are erroneously regarded to be of ectodermal origin; these ducts are further stated to fuse posteriorly to form an unpaired duct. Three genital invaginations from sterna 7, 8, and 9 may furnish the remainder of the unpaired common oviduct, the eighth becoming confluent with the seventh, whose opening to the exterior closes down (Orthoptera), and the ninth with the eighth, its opening closing (Coleoptera, &c.), the common duct thus formed by the confluence of the invaginations joining the unpaired portion of the paired oviducts. Accessory diverticulæ

from these invaginations give rise to the spermatheca and various accessory glands, and, as their number and origin varies, these structures cannot be homologized in the different groups even if they have a similar function. Christophers (1928) and Christophers and Barraud (1926) have made a valuable contribution to our knowledge of the development of the genital ducts in Diptera. These workers show that the genital cavity is morphologically different from the common oviduct. The latter originates as an invagination from the hinder part of the eighth sternum and grows anteriorly to the seventh sternum, where it is joined by the mesodermal oviducts. Between sterna 8 and 9 or within the eighth, the spermathecal invagination originates as an independent invagination posterior to the common oviduct. A third invagination from the ninth sternum, the 'caecus' gives rise to the accessory glands (mucus gland in Mosquito, collateral gland in *Phlebotomus*). By approximation these originally separate invaginations come to open later into a common genital infolding, the genital atrium. Singh-Pruthi (1924 c), working on *Tenebrio* (Coleoptera), finds that in this group also the mesodermal paired oviducts end on the seventh sternum. Further, that the 'uterus' originates as an unpaired hypodermal invagination from the hind-margin of the eighth sternum, the spermatheca and accessory gland from an invagination behind the ninth. The 'uterus' invagination later becomes confluent with the spermathecal invagination. Singh-Pruthi concludes that the spermatheca primitively opens behind the ninth sternum in insects, the 'uterus' on the eighth. Although finding the 'uterus' invagination to be unpaired from the beginning, he takes it to correspond to the paired ectodermal ejaculatory ducts, the existence of which he postulates in the male of Homoptera (1924 a) and Coleoptera (1924 b), and to represent two coalesced ducts. Recently George¹ has again fully treated the genital ducts and accessory organs. Working on *Philaenus* (Homoptera) and *Agriion* (Zygoptera), and

¹ Dr. C. J. George has courteously allowed me to refer to the manuscript of his paper, which has since been published in 'Quart. Journ. Micr. Sci.', vol. 72, pp. 447-85.

reviewing earlier work, this author arrives at an interpretation of the morphology of the internal genital structures which differs from that of Singh-Pruthi in several respects. He finds that there is little or no evidence for a paired origin of the common oviduct and suggests that in some insects it opened primitively behind the seventh sternum, a condition exhibited by some adult insects and during development by Homoptera. With Berlese he recognizes a tendency to shift the gonopore posteriorly. According to George, however, the first evolutionary stage is the formation of a groove from the seventh to the eighth sternum which closes and becomes tubular, the gonopore being located on the eighth sternum, e. g. Homoptera. A further stage is furnished by a groove from the eighth to the ninth sternum which becomes tubular and results in the oviducal opening being placed on the ninth (Lepidoptera). In Zygoptera George finds no evidence that the common oviduct opened primitively between sterna 7 and 8, this organ being derived partly from an invagination from the eighth sternum (which is later extended on to the ninth), and partly from the mesodermal oviducts. The evolutionary history of the accessory structures, e. g. spermatheca, &c., is left an open question by George, who, however, inclines to Berlese's view that they may be developed from invaginations from the seventh, eighth, and ninth sterna.

III. MATERIAL AND METHODS.

The nymphal and adult stages of *Locustana pardalina*, *Blattella germanica*, and *Forficula auricularia* were obtained by breeding these insects in the laboratory. *Colemania sphenarioides* and additional material of *Locustana* were collected in the field. Comparative studies were made on nymphs and adults of *Xiphidion* sp. (Tettigoniidae), *Gryllus domesticus* L. (Gryllidae), *Dixippus morosus* Br. (Phasmidae), adults of *Mantis* sp. (Mantidae), *Periplaneta americana*, and *Blatta orientalis* L. (Blattidae), all Orthoptera, and young and adult stages of the Thysanuran *Petrobius carpenteri* Bagnall (Machilidae).

Good fixation was given by Carls's mixture (see Tillyard,

'Biology of Dragonflies', 1917) and Carnoy II (see Lee, 'Microtomist's Vade-Mecum'), used cold from 8-24 hours according to the size of the insect to be fixed. In the older stages, puncturing or decapitation was necessary to ensure satisfactory penetration. After washing thoroughly in 70 per cent. alcohol, the material was prepared for sectioning by the conventional methods of paraffin-embedding, using cedar-wood oil as a clearing agent. Paraffin-embedded material gave general satisfaction, sections being cut 6-10 μ thick. For difficult material, e.g. advanced stages of *Locustana* and *Forficula*, double embedding (celloidin and paraffin) was necessary. Good preparations were obtained by staining in Ehrlich's acid haematoxylin followed by Biebrich scarlet and in borax carmine differentiated in picro-indigo-carmin (alcoholic). Mallory's triple stain also gave useful preparations, the chitinous parts being vividly differentiated.

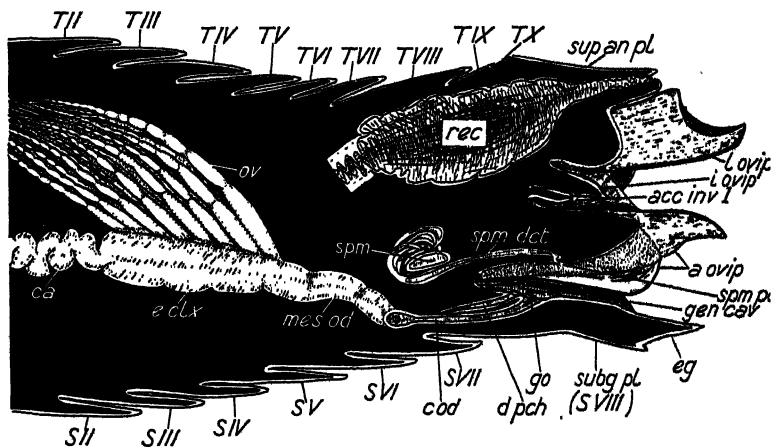
IV. OBSERVATIONS ON *LOCUSTANA* AND *COLEMANIA*.

(a) Adult Organs.

The genitalia and the genital duct system of *Locustana* are shown in Text-fig. 1. The general plan of the reproductive organs is similar in *Colemania*, and the ensuing description of these based on *Locustana* applies to *Colemania* also. The enlarged eighth sternum constitutes the subgenital plate (*subg pl*), its reflexed inner surface forming the floor of the genital cavity (*gen cav*) and being in part modified into a median egg-guide (*eg*). The two large anterior ovipositor lobes or ventral valves (*a ovip*) are joined to the membranous hind margin of the subgenital plate. Above these are the two pairs of appendages of the ninth sternum; a pair of lateral ovipositor lobes or dorsal valves (*l ovip*) and a pair of small, inner ovipositor lobes or valves (*i ovip*) between them. The gonopore or vulva (*go*), the opening of the common oviduct (vagina, uterus), is on the inner surface of the subgenital plate and ventral to the anterior ovipositor lobes. Immediately above it is a blind pouch (*d pch*), an invagination of the genital cavity, of uncertain function. The spermathecal pore (*spm po*) is in a groove in the

membrane stretching between the ventral bases of the anterior ovipositor lobes and is strengthened by a small chitinous plate for receiving the tip of the penis ; it does not open into the common oviduct but directly into the genital cavity. Between the inner

TEXT-FIG. 1.



Right half of the abdomen of an adult female of *Locustana* showing the reproductive organs. For lettering see p. 81.

ovipositor lobes is the opening of an unpaired, vestigial accessory gland.

A short, median common oviduct (*c od*) passes inwards from the gonopore and reaches to the anterior margin of the eighth sternum where the two oviducts (*mes od*) open into it. The oviducts diverge and run anteriorly and dorso-laterally. In the third to fifth abdominal segments each is dilated into an egg-calix (*e clx*) and receives the tubules of an ovary on its mesal margin ; beyond the ovary each oviduct extends into the thorax as a convoluted gland, the so-called 'boyau-calicial' (*ca*), which produces the 'froth-substance' accompanying the eggs when they are laid. The spermathecal pore leads into a horizontal spermathecal duct (*spm dct*) which soon forms a much-coiled structure ; on unravelling this the duct is seen to end by forking into a spermatheca and a spermathecal gland. The opening between the inner ovipositor lobes on the ninth sternum leads

into a short, straight, non-glandular invagination (*acc inv I*), hitherto undescribed, which represents a vestigial accessory gland, as will be shown later (VII (d), p. 63).

(b) Development of the Genitalia.

In late embryos, and in the early first instar nymphs of *Locustana*, the rudiments of two pairs of ovipositor lobes are plainly recognizable; an anterior, blunt-pointed pair of lobes on the hind margin of the eighth abdominal sternum, and a more sharply pointed, better-developed pair on the hind margin of the ninth. The two pairs of lobes arise in an identical manner from their respective sterna; ventrally each pair is separated by a suture from its sternum and each attaches along the whole width of its sternum. In cross-section the lateral attachment of the ovipositor lobes of a pair to their sternum is plain. Figs. 1 and 3, Pl. 2, respectively show cross-sections through the lateral (*l ovip*) and the anterior (*a ovip*) lobes of an early first instar nymph. Mesally, the two lobes of a pair become united near their bases leaving only a small groove ventrally (fig. 1, *gr 1*, fig. 3, *gr 2*). In newly hatched nymphs the bases of the anterior lobes are already slightly overgrown laterally by the eighth sternum, the first indication of the future enlargement of this plate into the subgenital plate with the consequent formation of a genital cavity. Similar conditions obtain in young first instar nymphs of *Colemania*, and in this form also it is clear that the lobes from the eighth and ninth sterna are serially homologous.

In the early second instar nymphs of *Locustana* and *Colemania* two small lobe-like outgrowths (fig. 8, *i ovip*, Pl. 2) arise from the mesal bases of the ovipositor lobes (*l ovip*) of the ninth sternum, and the ventral groove (*gr 1*) is now bounded by the inner margins of these outgrowths. There are no outgrowths from the lobes of the eighth (fig. 9, *a ovip*, Pl. 2) but their hypoderm is noticeably thickened mesally. The rudiments of the three pairs of ovipositor lobes of the adult are now present. The pair of lobes on the eighth sternum will develop into the ventral valves, the lateral lobes on the ninth

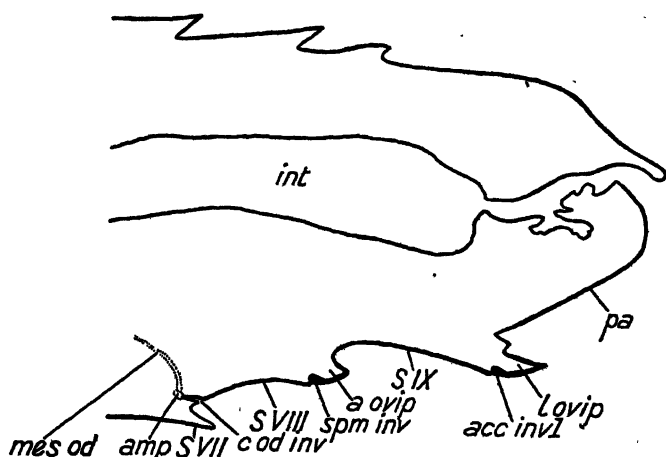
into the dorsal valves ; the inner pair on the ninth give rise to the inner valves.

During development to the adult the following changes take place. In the instars succeeding the second, the eighth sternum proceeds to enlarge and to overgrow the anterior ovipositor lobes until in the last (fifth) nymphal instar it is definitely established as a true 'subgenital' plate. In the third instar a small median tract of thickened hypoderm on the inner surface of the eighth sternum marks the beginning of the formation of the egg-guide. In the fifth instar this structure is well developed and has assumed the appearance of the adult organ. Mention may further be made of a pair of small secondary pockets (figs. 16, 17, and 18, *l pch*, Pl. 3) on either side of the egg-guide which are at this stage differentiated on the inner surface of the subgenital plate. The ovipositor lobes of the ninth sternum increase considerably in length, covering the inner lobes of the ninth sternum in the fourth instar, while in the fifth instar their extremities are only slightly anterior to those of the lateral ovipositor lobes of the ninth. In the last instar the superficial demarcation of each anterior ovipositor lobe by means of sutures into a compound 'basivalvula' of three sclerites and a 'shaft' is plain. The ninth sternum remains small and in the fifth instar only two lateral plates remain. The lateral ovipositor lobes lengthen considerably after the second instar, but the inner lobes remain small and fuse basally, only their tips remaining free. The extension of the eighth sternum posteriorly together with the reduction of the ninth and its retraction within the eighth results in the lateral and inner ovipositor lobes being situated dorsal to the lobes of the eighth and also in the formation of a large genital cavity covered above by the ovipositor lobes and floored below by the subgenital plate. Fig. 45, Pl. 4, illustrating a longitudinal section through a nymph of *Colemania* 22 mm. long, corresponding to the fourth instar of *Locustana*, and figs. 13-20, Pl. 3, illustrating selected cross-sections from a series proceeding headwards in a fifth instar nymph of *Locustana*, show the relations of the genitalia, the subgenital plate enclosing a genital cavity, &c.

(c) Development of the Genital Ducts and Accessory Organs.

In late embryos of *Locustana* a short, anteriorly growing, hypodermal inpushing, the common oviduct invagination, is present where the intersegmental membrane of the seventh abdominal sternum joins the eighth sternum, and runs out as a groove on to the eight sternum. Lying internally on either

TEXT-FIG. 2.



Schematic longitudinal section through the abdomen of an early first instar nymph of *Locustana* showing the rudimentary genital invaginations. The laterally placed right mesodermal oviduct (*mes od*) and ampulla (*amp*) are shown in dotted outline. For lettering see p. 81.

side of this inpushing are the ampullae, the slightly dilated ends of the mesodermal oviducts which stretch anteriorly to the ovaries as extremely minute, solid cords composed of lengthened cells. Essentially similar conditions prevail in the early first instar, but the common oviduct now opens on the extreme anterior part of the eighth sternum (Text-fig. 2, *cod inv*); its dorsal half extends posteriorly from its opening as a very short groove (fig. 5, *c od gr*, Pl. 2) which is open ventrally. Fig. 6, Pl. 2, of a cross-section a few sections anteriorly to that shown

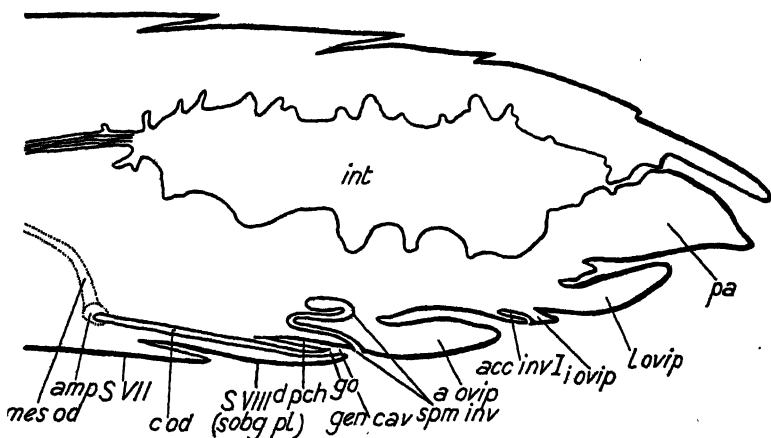
in fig. 5, shows the completely tubular common oviduct invagination (*c od inv*). As in the embryo the invagination ends blindly (fig. 7, *c od inv*, Pl. 2) between the two ampullae (*amp*) anteriorly, near the hind margin of the seventh sternum. The ventral groove (fig. 3, *gr 2*, Pl. 2) described above between the ovipositor lobes on the eighth sternum becomes tubular anteriorly and extends internally as a very short hypodermal invagination (fig. 4, Pl. 2, and Text-fig. 2, *spm inv*). This invagination, which is entirely independent of the common oviduct invagination, represents the rudiment of the spermatheca. The groove (fig. 1, *gr 1*, Pl. 2) between the ovipositor lobes on the ninth sternum gives rise to an invagination in the same way, the rudiment of the accessory genital invagination on the ninth (fig. 2, Pl. 2; Text-fig. 2, *acc inv 1*).

Newly hatched *Colemania* nymphs exhibit features similar to those presented to the corresponding stage of *Locustana*. There are also present three distinct invaginations, the spermathecal and the accessory invaginations between the lobes on the eighth and ninth sterna respectively, and the common oviduct invagination. As in *Locustana* the latter is unpaired and opens on the extreme anterior part of the eighth sternum, practically between sterna 7 and 8.

During further development important changes take place. *Colemania* resembles *Locustana* so closely that the two forms may be considered together. Considering the common oviduct invagination it is found that during the first instar its opening undergoes a very significant change of position. The mouth of the invagination, which is at the extreme anterior margin of the eighth sternum in the early first instar (Text-fig. 2, *c od inv*), is near the hind margin of the sternum towards the end of the instar. This 'shifting' of the gonopore appears to take place as follows. The short ventral groove (fig. 5, *c od gr*, Pl. 2) into which the common oviduct runs out is extended posteriorly, its anterior portion closing ventrally and becoming tubular as it proceeds towards the hind margin of the sternum. The common oviduct is thus extended and its opening, the gonopore, carried more and more posteriorly. In the newly

moulted second instar the common oviduct (fig. 11, *c od*, Pl. 2) runs out into a groove continuous with the genital cavity (*gen cav*) which is being formed at the hind margin of the eighth sternum by the sternum overgrowing the bases of the anterior ovipositor lobes (fig. 10, *b- a ovip*, Pl. 2). The common oviduct (*c od*) thus comes to open on the inner reflexed surface of the

TEXT-FIG. 3.



Schematic median longitudinal section through the abdomen of a nymph of *Colemania* 14 mm. long (corresponding to the second instar of *Locustana*) showing the relations of the genital invaginations to one another at this stage. The laterally placed right mesodermal oviduct (*mes od*) and ampulla (*amp*) are shown in dotted outline. For lettering see p. 81.

eighth sternum (subgenital plate), as is shown in Text-fig. 3. Part of the genital cavity is carried anteriorly for a short distance as an invagination above the common oviduct, the rudiment of the 'pouch' noted in the adult (Text-fig. 3; fig. 12, *d pch*, Pl. 2). The intermediate stages of the progression of the common oviduct from the seventh sternum to the hind margin of the eighth has not been followed in *Colemania*, but this probably takes place as in *Locustana*.

Further development shows few significant changes. In the third instar the common oviduct still ends blindly between the ampullae, now slightly swollen and with a distinct lumen.

In the oviducts also a small lumen is just discernible. In the fourth instar the now wider and larger common oviduct is surrounded by connective tissue and muscle and the ampullae of the oviducts with their clear lumina are about to open into it. The blind pouch above the common oviduct is better developed and muscles attach to its tapering end. In the last instar the large oviducts (fig. 22, *mes od*, Pl. 3) open into the wide common oviduct (*c od*) and their lumina are continuous.

The spermathecal invagination from between the ovipositor lobes of the eighth sternum develops comparatively rapidly and extends anteriorly. In the second instar it becomes bent upon itself and again towards the end of the instar (figs. 10, 11, Pl. 2; Text-fig. 3, *spm inv*). In the fourth and fifth instars it is a long tube coiled anteriorly (fig. 19, *spm inv*, Pl. 3; fig. 45, Pl. 4). It may be emphasized that, as in the adult, during development there is no connexion between the common oviduct and the spermathecal invagination; the spermathecal invagination opens not into the roof of the common oviduct but into the genital cavity when this is formed.

The accessory genital invagination between the ovipositor lobes on the ninth (Text-fig. 2; fig. 4, *acc inv 1*, Pl. 2) develops slowly in comparison with its serial homologue on the eighth, the spermathecal invagination. It increases slowly in length through the different instars up to the fifth, when it is a short tube extending anteriorly and slightly bent upon itself at its anterior end as if to start coiling (see Text-fig. 3; fig. 15, *acc inv 1*, Pl. 3; fig. 45, Pl. 4). Even in the adult this structure is vestigial (Text-fig. 1, *acc inv 1*).

The relations of the genital invaginations to one another and to the ovipositor lobes, subgenital plate, and genital cavity in late nymphal stages are shown by fig. 45, Pl. 4, illustrating a longitudinal section through a nymph of *Colemania* corresponding to the fourth instar of *Locustana*, and by figs. 13-22, Pl. 3, illustrating selected cross-sections from a series proceeding headwards in a fifth instar nymph of *Locustana*.

V. OBSERVATIONS ON BLATTELLA.

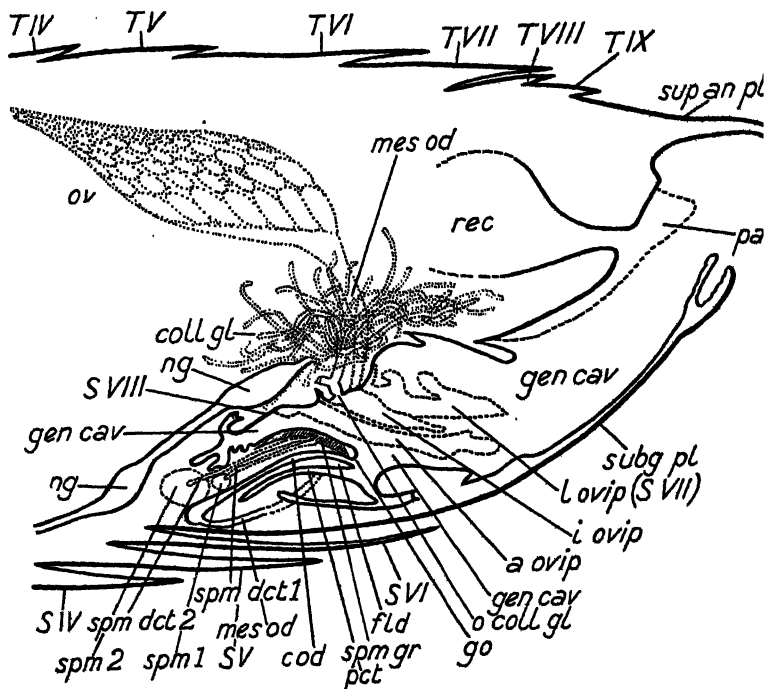
(a) Adult Organs.

The reproductive organs are shown below in Text-fig. 4 in schematic longitudinal section and in figs. 51-4, Pl. 4, illustrating selected cross-sections. The enlarged seventh sternum provides the subgenital plate (*subg pl*), its inner reflexed surface forming the floor of the huge genital cavity (*gen cav*) which is bounded dorsally by the eighth sternum, the ovipositor lobes, and the paraprocts. The inner surface of the subgenital plate is strikingly modified, having to carry the ootheca; it is thrown into folds and fold-like invaginations possessing areas of glandular cells (figs. 51-5, *gl c*, Pl. 4). In cross-section, two main lateral folds (fig. 51, *l fld*, Pl. 4) are seen rising up on either side of the ovipositor lobes situated dorsally on the roof of the genital cavity. The three pairs of ovipositor lobes are reduced and almost entirely membranous. The eighth sternum is reduced and largely dechitinized, while its appendages, the anterior ovipositor lobes or ventral valves (*a ovip*), are closely apposed to the lateral (*l ovip*) and inner (*i ovip*) ovipositor lobes of the ninth sternum, and connected with both these pairs by a tongue and groove arrangement (see fig. 51, Pl. 4). The ninth sternum is reduced to two small lateral areas. For a detailed description of the chitinized portions remaining of the eighth and ninth sterna see Wille (1920, pp. 86-91).

The ovaries are situated dorso-laterally in the fourth to the seventh abdominal segments. Leading from the ovaries in the seventh segment are the two short oviducts (figs. 52, 53, 54, *mes od*, Pl. 4) which proceed ventrally and anteriorly to open into the common oviduct (Text-fig. 4, *c od*). The common oviduct is short and opens by means of the gonopore (Text-fig. 4, *go*) on a raised fold (*fld*) of the floor of the genital cavity very near its anterior end. This raised fold is due to a pocket (Text-fig. 4; figs. 53, 54, *pct*, Pl. 4) of the genital cavity extending beneath the part carrying the gonopore. The gonopore is thus on the reflexed inner surface of the seventh sternum, the seventh intersternal or so-called intersegmental membrane.

Four small bulbous bags lying internally in the body-cavity, two on each side of the common oviduct (Text-fig. 4, *spm 1* and 2), serve as spermathecae, as sections of females which had copulated show all four to be filled with spermatozoa. The

TEXT-FIG. 4.



Schematic median longitudinal section through the abdomen of a newly moulted adult female of *Blattella* showing the reproductive organs. Parts not in the median plane are shown in dotted outline. For lettering see p. 81.

spermathecae open directly into the genital cavity and not into the common oviduct (vagina), as is often inaccurately stated. A short duct leads from each (fig. 54, Pl. 4; Text-fig. 4, *spm dct 1* and 2) to open into a short dorsal groove (figs. 53, 54, *spm gr*, Pl. 4) on the fold carrying the gonopore, the two ducts of a side tending to open confluent. The spermathecal openings are thus on the floor of the genital cavity, the seventh

intersternal membrane—an aberrant and secondary condition, as will be shown later—anterior (morphologically posterior) to the gonopore. Posteriorly the groove (*spm gr*) leading from the spermathecal openings runs out on to the gonopore, furnishing a guiding passage for the sperms. A profuse mass of glandular tubes (Text-fig. 4; figs. 51–4, *coll gl*, Pl. 4), the accessory or colleterial glands, open on the ninth segment (not intersegmentally as stated by Wille 1920) ventrally between the bases of the inner ovipositor valves. The glands are paired, the tubules of a side leading into a short common duct which joins its fellow from the other side to open by a common pore (fig. 52, *o coll gl*, Pl. 4).

(b) Development of the Genitalia.

In first instar nymphs of *Blattella* the sterna of the genital segments are readily visible from below; the seventh sternum is normal, the eighth is to a large extent retracted within the seventh, while the ninth is divided posteriorly by a short median fissure from the hind margin into two lobes (fig. 23, *r-l ovip*, Pl. 3) which each bear a stylus laterally (*b-st*). As Wille (1920) states that the sexes cannot be distinguished externally until the last instar, it is perhaps worth mentioning that this fissure on the ninth sternum enables one to recognize the female from the earliest stages. During the succeeding instars, first the eighth and finally the ninth sternum becomes completely telescoped within the seventh sternum which, at the same time, gradually enlarges and becomes specialized as the sub-genital plate (fig. 46, Pl. 4; fig. 50, *subg pl*, Pl. 4), a capacious genital cavity being formed between the seventh sternum ventrally and the eighth and ninth sterna and paraprocts dorsally. The hypoderm of the floor of the genital cavity (formed by the inner surface of the seventh sternum, the seventh intersternal membrane) is early thickened. In the fourth instar the two lateral folds are evident (fig. 39, *lfd*, Pl. 4). In the next instar more folds and fold-like invaginations have made their appearance. In the following and last nymphal instar tracts of cells at the bases of these become specialized as glandular cells (figs. 47, 48, *glc*, Pl. 4).

The gonapophyses are not recognizable externally in the first instar. Sections of nymphs in this stage show that each lobe of the ninth sternum is thickened mesal to the styli (fig. 23, *r-l ovip*, Pl. 3), the first indication of the future lateral ovipositor lobes. A similar mesal thickened area can be seen on the hind margin of the eighth sternum (fig. 23, *r-a ovip*, Pl. 3), and a median fissure soon appears which differentiates the two rudiments of the anterior ovipositor lobes. Apart from the initial absence of the fissure on the eighth sternum, there can be no doubt as to the similarity of origin of the anterior ovipositor lobes of the eighth and the lateral lobes of the ninth. That these structures correspond serially is especially evident in later instars when these structures are better differentiated (compare on Pl. 3, fig. 26, *l ovip*, with fig. 30, *a ovip*). It is true that in the late instars the tips of the anterior ovipositor lobes come to occupy a median position, but a section through their base compared with one through the base of the lateral ovipositor lobes of the ninth at once shows the homology of these structures (compare on Pl. 4, fig. 37 with figs. 39 and 40). In the third instar the inner ovipositor lobes of the ninth sternum are first indicated as thickenings on the mesal margins of the lateral ovipositor lobes; in later instars they bud forth as outgrowths from these (fig. 37, *i ovip*, Pl. 4). No outgrowths homologous with these are differentiated from the anterior ovipositor lobes of the eighth sternum. The eighth and ninth sterna do not develop into normal sternal plates. They are lightly chitinized in the early instars, and when they are retracted within the seventh sternum (subgenital plate) they become dechitinized and are represented by short, wide, transverse strips of membrane. By the last nymphal instar, the ninth sternum is practically crowded out or absorbed mesally by the bases of the lateral and inner ovipositor lobes. The eighth sternum remains a well-defined membranous area lining the anterior part of the roof of the genital cavity. In the adult this area becomes chitinized in part, chiefly marginally. The lateral ovipositor lobes of the ninth sternum develop from the sternum mesal to the styli and do not carry these structures apically as they are said to do in some insects,

e.g. Odonata. The styli are lost during the moult from the fifth into the last nymphal stage.

(c) Development of the Genital Ducts and Accessory Organs.

In the newly hatched and older first instar nymphs, the ampullae of the mesodermal oviducts can be seen resting beneath the hypoderm of the posterior part of the seventh sternum where the intersternal (intersegmental) membrane attaches to the eighth sternum. They have a small lumen and represent flattened structures (fig. 25, *amp*, Pl. 3). The mesodermal oviducts are continued from the ampullae anteriorly and dorsally to the ovaries as solid cords of cells. No trace of the common oviduct invagination can yet be made out. In the next stage, however, the rudiment of this organ is furnished by an unpaired hypodermal invagination which sinks in from the slightly thickened floor of the genital cavity, i. e. from the seventh intersternal membrane (fig. 32, *cod inv*, Pl. 3). In succeeding instars the common oviduct increases in size and length, its anteriorly growing end resting above the ampullar ends of the mesodermal oviducts (fig. 42, *cod inv*, Pl. 4). In the fifth instar the common oviduct is provided with a clear lumen into which the ampullar ends of the paired oviducts open by the breaking down of the intervening walls (see fig. 49, *cod inv*, *mes od*, Pl. 4, from a sixth instar nymph); the paired oviducts now have a well-defined lumen throughout their length. A secondary invagination (fig. 46, Pl. 4; figs. 48, 50, *pct*, Pl. 4) sinks in beneath the common oviduct so that in the adult it traverses and opens on a fold (Text-fig. 4; fig. 53, *fld*, Pl. 4) of the genital cavity floor. In the fourth instar two minute hypodermal invaginations sink in on each side of the mid-line from the hypoderm of the genital cavity floor, immediately anterior (morphologically posterior) to the common oviduct invagination (figs. 43, 46, *spm inv 1*, Pl. 4). Immediately anterior to these again is a similar pair of invaginations (figs. 44, 46, *spm inv 2*, Pl. 4). These two pairs of independent invaginations represent the rudiments of the four spermathecae. The spermathecal

rudiments enlarge (see fig. 50, Pl. 4, of a fifth instar nymph) to form four ingrowing tubes whose ends dilate in the last instar into the four bulbous bags, a pair on each side of the common oviduct (figs. 48, 49, *spm 1* and *2*, Pl. 4), the anterior pair being the larger. A small dorsal groove has now formed along the mid-line of the fold carrying the gonopore. The spermathecal ducts open separately into this with a tendency, however, for the pair of a side to open confluent into this groove (fig. 54, *spm gr*, Pl. 4). In the second instar, a small unpaired invagination (fig. 28, *acc inv 1*, Pl. 3) arises on the ninth sternum anteriorly from the groove (fig. 27, *gr 1*, Pl. 3), between the rudimentary lateral ovipositor lobes and this is the rudiment of the colleterial gland. When the inner ovipositor lobes are differentiated from the inner margins of the lateral ovipositor lobes on the ninth sternum, the groove comes to lie between these (fig. 38, *gr 1*, Pl. 4) and thus also the opening of the accessory invagination, as in the case of the corresponding accessory invagination in *Locustana* and *Colemania*. While but a short invagination, the colleterial rudiment gives rise to two lateral diverticulae (fig. 29, *acc inv 1*, Pl. 3), and these grow and give rise to many diverticulae. Some of these again fork and the paired colleterial gland of the adult is formed. A similar invagination arises from a groove (fig. 31, *gr 2*, Pl. 3) at the bases of the anterior ovipositor lobes on the eighth sternum. This invagination, which corresponds to the spermathecal invagination of *Locustana* and *Colemania*, remains rudimentary, and in the fourth instar is but a short invagination (figs. 41, 42, *acc inv 2*, Pl. 4). In the adult there is no trace of this invagination, a median tract of enlarged glandular cells marking its position (fig. 53, *s gl*, Pl. 4).

VI. OBSERVATIONS ON FORFICULA.

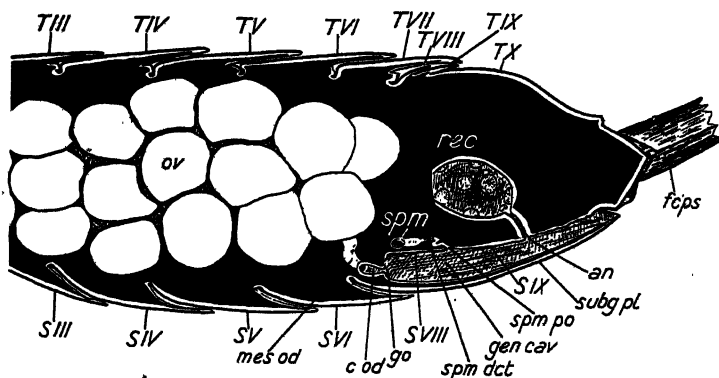
(a) Adult Organs.

Text-fig. 5 illustrates the reproductive organs of *Forficula*. As in *Blattella* the seventh sternum of the abdomen is enlarged to serve as a subgenital plate (*subg pl*). The reduced

eighth and ninth sterna are covered below by the subgenital plate and are lightly chitinized; the median area of each is membranous. No ovipositor valves are borne by the eighth and ninth sterna. A genital cavity (*gen cav*) is enclosed between the subgenital plate below and the eighth and ninth sterna above.

The ovaries in females about to oviposit occupy practically the whole of the abdomen from the second to the seventh seg-

TEXT-FIG. 5.



Right half of the abdomen of a gravid adult female of *Forficula* showing the reproductive organs. For lettering see p. 81.

ments. Two short oviducts (*mes od*) emerge from the ovaries posteriorly and pass downwards to open into the common oviduct (*c od*) at the anterior margin of the seventh sternum. The common oviduct is very short and opens into the genital cavity where the inner surface of the subgenital plate joins the eighth sternum; morphologically the gonopore (*go*) is on the posterior membranous part of the seventh sternum, the intersegmental membrane. Situated posteriorly in the median membranous area of the eighth sternum is the spermathecal pore (*spm po*); from it a thin, coiled duct leads into the bent spermatheca (*spm*) lying internally on the sternum. As in *Locus-tana*, *Colemania*, and *Blattella*, the spermathecal pore is distinct from the gonopore or egg-laying opening and opens directly into the genital cavity and not into the common

oviduct. Unlike these insects, *Forficula* does not possess a genital invagination on the ninth sternum.

(b) Development of the Genital Cavity, Genital Ducts, and Spermatheca.

The extension of the seventh sternum as a subgenital plate, and the retraction of the eighth and ninth sterna within it, with the consequent formation of a large genital cavity, takes place gradually during nymphal development. In the first three instars sterna 7, 8, and 9 are normal. When the last nymphal instar, the sixth, is reached, the eighth sternum is almost completely retracted within the seventh. In the adult the seventh sternum covers the eighth and ninth sterna and the genital openings and forms the subgenital plate. There are no indications of gonapophyses during nymphal development.

In the second instar two genital invaginations are evident. A short hypodermal invagination originates between the seventh and eighth sterna from the intersternal membrane and extends anteriorly, ending blindly; it represents the rudiment of the common oviduct (fig. 35, *cod inv*, Pl. 3). The posterior ends of the minute, solid oviducts, which come from the ovaries situated dorsally in the fourth and fifth abdominal segments, are closely applied to the blind end of the common oviduct invagination in the seventh segment, one on each side (fig. 36, *amp*, Pl. 3). The ends of the oviducts are not dilated into ampullae to the same extent as in *Locustana* and *Colemania*. Heymons (1895 a) states that these ampullae terminate in the tenth segment and this has been widely quoted. As is evident from above, the ampullae are in the seventh segment, as in all other insects. Heymons has in fact stated that this observation was erroneous (1901, footnote, p. 188), but this has been overlooked by practically all subsequent authors. Originating from the inner surface of the eighth sternum is the second invagination, the rudiment of the spermatheca (fig. 34; *spm inv*, Pl. 3). Fig. 33 (Pl. 3) illustrates the common oviduct invagination (*cod inv*) and the spermathecal or accessory genital

invagination (*spm inv*) of a third instar nymph in longitudinal section.

These invaginations undergo little change during further development. The common oviduct invagination becomes wider and deeper during its development through the instars up to the last, when it is a short wide duct. The mesodermal paired oviducts increase in diameter and have acquired a lumen throughout their length in the last instar. They now open into the common oviduct. During the instars following the second the spermathecal invagination increases in length and becomes coiled (fig. 33, *spm inv*, Pl. 3). In the last instar its terminal portion is dilated into the spermatheca proper, the remainder of its length serving as the spermathecal duct. There are no traces of an invagination from the ninth sternum during development.

VII. DISCUSSION.

A. Genitalia.

It has been generally accepted up to now (Verhoeff and others) that in pterygote insects the anterior ovipositor valves of segment 8 are serially homologous with the inner ovipositor valves of segment 9. Two recent workers on the genitalia of Orthoptera, viz. Walker (1918, 1922) and Chopard (1920), also entertain this view. My observations on *Locustana*, *Colemania*, and *Blattella* indicate that it needs revision. In these insects the ovipositor valves are first represented by two pairs of lobe-like outgrowths on the eighth and ninth sterna. The manner and place of origin of these rudiments leaves no doubt that they are serially homologous. Later the lobes on the ninth give rise to a pair of outgrowths on their mesal margins. The lateral structures on the ninth develop into the lateral ovipositor valves, the mesal pair into the inner valves. No mesal outgrowths are differentiated from the lobes of the eighth sternum and they develop directly into the anterior ovipositor valves. From the above considerations I must therefore regard the anterior ovipositor lobes of Orthoptera as corresponding

with the lateral valves of the ninth, with gonocoxites from which no telopodites are differentiated.

Wheeler (1893) has already recognized that the rudiments of the anterior and lateral ovipositor valves in Orthoptera are serially homologous, but later workers, like Verhoeff and others, have overlooked this, and the idea that the anterior ovipositor valves in Orthoptera and other pterygotes represent telopodites appears to have been suggested by the fact that they correspond functionally with the telopodites (ovipositor lobes) of Machilidae and Lepismidae borne by the gonocoxites of segment 8. In this respect a comparison of the origin of the ovipositor lobes in young Orthoptera and in the young stages of the Machilid *Petrobius carpenteri* Bagnall is instructive, and clearly shows that the anterior ovipositor valves of Orthoptera do not correspond morphologically with the ovipositor lobes of Machilidae but with the gonocoxites of these insects. Walker and Chopard, although they include accounts of the development of the ovipositor lobes in their studies, have failed to observe that the mode of origin of the anterior ovipositor valves from the eighth sternum is similar to that of the lateral valves from the ninth and entirely dissimilar to that of the inner valves between these. In most Orthoptera the anterior valves are soon overgrown by the eighth sternum to a greater or less extent, so that it is difficult to determine externally that the bases of the anterior valves also connect laterally with their sternum as do the lateral valves. As Walker and Chopard do not appear to have studied sections of young nymphs but to have relied on whole preparations and chitin mounts, the true morphological nature of the anterior valves escaped their notice.

The question arises whether homologues of the anterior ovipositor lobes of *Thysanura*, i.e. serial homologues of the inner valves of the ninth segment occur in Pterygota. This appears to be the case, especially in the light of the recent work of George.¹ In Homoptera George shows that the ovipositor is first represented by two pairs of serially homologous rudiments on the eighth and ninth sterna. Both pairs later give rise to a

¹ See footnote, p. 31.

pair of mesal processes. The lateral processes of the eighth, which correspond to the rudiments of the lateral valves of the ninth, grow slowly and form the 'basivalvula' of the compound anterior ovipositor valves of the adult, the mesal processes between these, which correspond to the inner valves of the ninth sternum, enlarging rapidly to ultimately form the major portion of the anterior valves. In the Orthoptera the anterior valves of the adult are also compound structures consisting of a so-called 'basivalvula' of three sclerites and a 'shaft'. But this division appears very late in ontogeny and is to be regarded as secondary, for it is only a superficial demarcation and in no way comparable to the primary division into two parts of the anterior ovipositor valve rudiments of the Homoptera. In Zygoptera, George describes and figures an inner pair of processes between the anterior ovipositor valves. These, I take it, probably correspond to the anterior ovipositor valves of Machilidae and represent additional structures not differentiated in Orthoptera, while the anterior ovipositor valves which bear them represent the gonocoxites of the eighth segment.

It thus appears that the anterior ovipositor valves of Pterygota (where such structures occur) are not always strictly identical morphologically as has been assumed hitherto. In Homoptera the inner ovipositor valves or telopodites of the eighth segment are greatly enlarged and form the greater part of the anterior valves, the gonocoxites being reduced to small plates. In Zygoptera (Odonata) and Orthoptera the anterior ovipositor valves represent gonocoxites. These in Zygoptera bear vestigial inner ovipositor valves or telopodites; in Orthoptera no such structures are differentiated, their probable remnants having been completely incorporated within the coxites.

B. Genital Ducts and Accessory Organs.

In descriptions of the genital ducts, the same name has often been applied to parts which are morphologically distinct, so that a confused terminology has arisen which has not only tended to obscure an understanding of these organs but has often led to

needless discussion. Therefore it seems desirable before passing to a discussion of these organs to define briefly how certain terms are used in the present paper. The 'common oviduct' is the terminal unpaired portion of the efferent system into which the paired mesodermal oviducts open and it corresponds to the 'vagina', 'uterus', 'vagina+uterus', 'vagina+common oviduct', 'common oviduct—vagina' of authors. It opens by means of the 'gonopore' ('vulva' of some authors) usually into a 'genital cavity' ('vagina', 'vagina' part, 'uterus' part, 'bursa copulatrix' of authors) generally formed by the telescoping of the terminal abdominal segments within a sternum (seventh or eighth mostly) specialized as a subgenital plate. The 'spermatheca' (receptaculum seminis of authors) is the reservoir in which the sperm is finally stored; its opening to the exterior is termed the 'spermathecal pore'.

(a) The Common Oviduct.

Some difference of opinion still exists regarding the manner of origin of the common oviduct. Whereas most workers describe it as being formed only from the hypoderm, others contend that part of it is of mesodermal derivation, e.g. Wheeler (1893), George, a view to which Imms subscribes in his text-book (1925). And while the common oviduct is generally held to be of unpaired origin, Nusbaum's belief that it is derived from a coalescence of paired rudiments is still tentatively expressed in some text-books, e.g. Deegener (1913-21), and has recently been promulgated anew by Singh-Pruthi (1924 c).

Heymons (1891, 1895 a) and Wheeler (1893) have shown conclusively in their embryological studies on Orthoptera that the mesodermal paired oviducts terminate in the seventh segment, their ends being usually enlarged into ampullae. As my studies deal with the origin and post-embryological development of the common oviduct and its fusion with the mesodermal oviducts, the entire developmental history of the genital ducts in this group is available. In *Blattella*, *Locustana*, and *Colemania* the common oviduct has been shown to originate as a median, unpaired hypodermal invagination from between

the seventh and eighth sterna, which extends inwards to between the ends of the mesodermal oviducts. In *Blattella* this hypodermal invagination gives rise to the common oviduct in its entirety; in *Locustana* and *Colemania* the common oviduct is formed by this invagination together with a posterior hypodermal extension, to be considered later. In these insects there can be no doubt that the entire length of the common oviduct is formed from the hypoderm; the paired mesodermal oviducts open into the common oviduct in a late nymphal stage and take no part in the formation of the unpaired terminal section of the efferent system. In the *Dermapteron* *Forficula* also, a hypodermal invagination from between the seventh and eighth sterna gives rise to the common oviduct into which the paired mesodermal oviducts come to open, one on either side. These facts are in contradiction to the statement of Wheeler (loc. cit., p. 125) that the 'uterus' is mesodermal. This author appears to imply that the unpaired terminal section of the genital ducts is derived partly from the mesodermal ampullae, the 'uterus' section, and partly from an ectodermal invagination, the 'vagina' (cf. also Imms, p. 150). As Wheeler's observations are restricted to the embryo and very young nymphs, and he did not follow the development of the common oviduct during the nymphal stages nor note the opening of the mesodermal oviducts into it, his assumption of a mesodermal 'uterus' cannot be regarded as resting on good evidence. The evidence presented by George (loc. cit.) for a mesodermal section of the common oviduct is better founded. This author finds that in early nymphs of *Agrion* (*Odonota*) the mesodermal oviducts fuse in the region of the eighth segment and extend posteriorly within it as a single cord upon which a hypodermal invagination from the eighth sternum rests. The common oviduct of the adult is formed by the mesodermal cord and the hypodermal invagination. In view of the fact that Heymons (1896 b) has described the mesodermal oviducts in young nymphs of *Agrion* as terminating in the seventh segment, as in all other insects, a condition which George has not linked up with the apparently later stages studied by him, the latter

author's recognition of a mesodermal section of the common oviduct must be accepted with reserve until confirmed.

Apart from the still doubtful case of *Odonata* the common oviduct is described as being formed only from the hypoderm in a number of insect Orders, e.g. Mallophaga (Strindberg), Homoptera (George), Lepidoptera (Jackson, Verson, and Bisson), Diptera (Bruël, Christophers, Christophers and Barraud), Hymenoptera (Seurat), Coleoptera (Singh-Pruthi). This condition therefore seems to be the general rule amongst the insects, there being little or no evidence for the views to the contrary.

The question of the paired or unpaired origin of the common oviduct, so long buried, has been raised again by Singh-Pruthi (loc. cit.). This worker postulates the existence of paired ectodermal ejaculatory ducts in male Homoptera and Coleoptera (1924 *a*, 1924 *b*), and believes that homologues of these are present in the female as described by Nusbaum. He homologizes the common oviduct with the paired ectodermal ejaculatory ducts of the male and contends that it has been derived from a coalescence of paired ectodermal ducts, even though he finds it to arise from an unpaired invagination. Singh-Pruthi further attempts to find support for his views in the Orthoptera by suggesting that a pair of evanescent 'ampullae' described by Wheeler (1893) represent the paired ectodermal rudiments of the 'uterus' (common oviduct). Wheeler found these ampullae to be situated in the tenth abdominal segment, but Singh-Pruthi dismisses this by stating that 'as regards the demarcation of the body-segments, Wheeler was not very particular', and holds that the structures in question 'should lie near the posterior margin of the eighth segment'. These and other remarks of Singh-Pruthi (loc. cit., p. 878) criticizing Wheeler's work are not backed up by any evidence of his own or of other workers. Moreover, Singh-Pruthi ignores the fact that Heymons (1895 *a*) fully confirmed Wheeler's observations in a number of Orthoptera. These ampullae are derived from mesodermal coelomic diverticulae and are restricted to a short period of embryonic life. The common oviduct invagination originates during the close of embryonic life or post-embryoni-

cally from an unpaired ectodermal invagination between the seventh and eighth segments, as Wheeler and Heymons have noted and I can fully confirm in the Orthoptera *Blattella*, *Locustana*, and *Colemania*, and in the Dermapteron *Forficula*. Nusbaum's observations, which in the female form the only support for Singh-Pruthi's restatement of that author's views, are thus rendered highly improbable in *Blatta*; in *Mallophaga* they have been shown incorrect by the work of Strindberg (1916 a). To conclude: in Orthoptera and Dermaptera, as in other orders of insects, e.g. Hymenoptera (Seurat), Homoptera (George), Lepidoptera (Jackson, Verson and Bisson), Diptera (Bruël, Christophers, Christophers and Barraud), there is no evidence whatsoever for a paired origin of the common oviduct, and the postulated corollary, the existence of primitive paired ectodermal oviducts in insects.

(b) The Primitive Position of the Gonopore in Orthoptera and Dermaptera.

The primitive situation of the gonopore or opening of the common oviduct in the Orthoptera (sens. lat.) has not yet been critically investigated, the usual situation in these insects on or within the posterior limits of the eighth sternum, e.g. *Locustidae*, *Tettigoniidae*, *Gryllidae*, *Phasmidae*, being tacitly assumed to represent the ancestral condition (Walker, Chopard, &c.). In *Blattidae* and *Mantidae* the gonopore was formerly incorrectly likewise located on the eighth sternum (Miall and Denny, 1886), but it has now been shown conclusively to be situated between the seventh and eighth sternum (strictly within the seventh intersternal membrane) by Denny (1898), Peytoureau (1895), Bordas (1909), Chopard (1920), Wille (1920), Vogel (1925), &c. To bring these two groups into line with the other Orthoptera, Chopard and Walker state that in their case the gonopore has experienced a secondary shifting anteriorly.

A comparison of the development of the common oviduct in *Blattella* (*Blattidae*) and in *Forficula* (*Forficulidae*) with that in the *Locustidae*, *Locustana* and *Colemania*, definitely indicates that the condition of the two first-mentioned

insects, where the common oviduct opens between the seventh and eighth sterna, is the more primitive, and that the gonopore on the eighth sternum in Locustidae is a later specialization. It has been shown above that in *Blattella* and *Forficula* the short common oviduct opens on the seventh intersternal membrane from an early nymphal stage right up to the adult. In *Locustana* and *Colemania* the opening of the common oviduct invagination is in late embryos between the seventh and eighth sternum—a condition similar to that of *Blattella* and *Forficula*. During development the oviduct is extended posteriorly along the eighth sternal plate, its opening finally becoming located on the inner reflexed surface of this plate. This recapitulation of a common oviduct invagination between the seventh and eighth sterna by the Locustidae indicates in no uncertain manner that their ancestral condition as regards the common oviduct and the gonopore was essentially similar to the condition still exhibited by Blattidae and Forficulidae, or in other words that the gonopore in ancestral Locustidae opened between the seventh and eighth sterna, the situation in present-day representatives being a secondarily acquired condition. Chopard and Walker's idea, therefore, that the gonopore has secondarily shifted anteriorly in some Orthoptera, must be abandoned in face of the ontogenetic evidence presented above; the reverse has taken place.

It remains to be seen if in the remaining Orthoptera the gonopore between the seventh and eighth sterna can be established as an ancestral condition. In Mantidae the gonopore in the adult opens between the seventh and eighth sterna (Chopard, Bugnion, 1923), although attempts have not been wanting to locate it on the anterior region of the eighth, e.g. Walker. Apparently, therefore, the Mantidae, like the Blattidae, have retained the primitive position of the gonopore. In the Tettigoniidae and Gryllidae, Chopard locates the gonopore between the seventh and eighth sterna describing the subgenital plate as being developed from the seventh intersternal membrane. Walker regards the subgenital plate as representing the anterior portion of the eighth sternum, the gonopore being definitely

situated within this sternum. I have examined nymphs and adults of *Conocephalus* (subg. *Xiphidion*) sp. (*Tettigoniidae*) and of *Gryllus domesticus* L. (*Gryllidae*), and must adhere to Walker's interpretation as the correct one. But Wheeler, in *Xiphidion* embryos, has shown that the common oviduct originates as an invagination between the seventh and eighth sterna, so that in the *Tettigoniidae* there can be no doubt that the common oviduct originally opened between the seventh and eighth sterna. The close similarity as regards the opening of the common oviduct, subgenital plate, &c., between the *Tettigoniidae* and the *Gryllidae* and the near affinities of these groups, makes it extremely probable that sections of very young nymphs will in the latter group also reveal the common oviduct opening between the seventh and eighth sterna. In the *Phasmidae* practically all workers state that the eighth sternum is modified to form a subgenital plate on the inner reflexed surface of which the common oviduct opens ventrally and anterior to the bases of the anterior ovipositor lobes. Chopard states that the gonopore is between sterna 8 and 9, but this is not quite accurate as I can state from an examination of a number of *Phasmids*; the gonopore opens on the inner reflexed surface of the eighth sternum. I have sectioned very young and older nymphs of *Dixippus morosus* and found the common oviduct to be well advanced, definitely opening on the inner surface of the subgenital plate. Earlier stages were not available for study, so it could not be determined whether a secondary shifting posteriorly of the gonopore takes place. I imagine that a study of late embryos will show this to be the case, considering the postulated affinities (*Handlirsch*) of the *Phasmidae* with the *Tettigoniidae*.

To sum up, it can be said that *Blattidae* and *Mantidae* exhibit a primitive *Orthopteroid* condition in retaining the gonopore between the seventh and eighth sterna. The *Dermapteron Forficula* likewise retains this primitive *Orthopteroid* condition. *Locustidae* recapitulate this condition during ontogeny and secondarily acquire the present-day situation of the gonopore on the eighth sternum. Evidence is adduced that *Tetti-*

goniidae also recapitulate the Blattid condition, and it is surmised on grounds of affinity that the Gryllidae and Phasmidae will be found to show a similar recapitulation.

(c) The Gonopore and Common Oviduct in Insects in General.

It is almost universally held that in the insects the primitive situation of the female gonopore is on the eighth sternum between or near the bases of the anterior ovipositor lobes. The hypothetical 'protentomon' was and is still characterized with this condition (Mayer, 1876; Handlirsch, 1908, 1913-25). Palmen's main thesis that the paired genital openings of the mesodermal oviducts in Ephemera represent a primitive condition for insects has not yet found general acceptance, due chiefly to its confusion with Nusbaum's imaginative hypothesis of primitive paired ectodermal ducts, which has been disproved time and again. His flirtation with the logical consequence of his thesis, viz. that some insects acquired a median unpaired ectodermal oviducal portion from the seventh intersternal membrane as a first departure from the ephemerid condition, has not been taken seriously. Korschelt and Heider's suggestion (1899, p. 350) that the posteriorly placed genital apertures may be secondary has likewise been disregarded. Now that it has been shown that the primitive location of the single gonopore in the Orthoptera is between the seventh and eighth sterna, Palmen's theses, based mainly on anatomical considerations become significant and deserve careful consideration also in the light of ontogenetic work. I believe that an incontrovertible case can be put forward in favour of double genital openings between the seventh and eighth sterna as an ancestral condition of insects from which, by the addition of an unpaired ectodermal common oviduct, the single gonopore condition of other insects is derived. Further, that the single gonopore was primitively located between the seventh and eighth sterna in practically all orders of insects.

Embryological and post-embryological research during the last decade has resulted in establishing certain facts with regard

to the female genital ducts whose fundamental significance has not been sufficiently appreciated up to now. I refer to the termination of the mesodermal oviducts on or near the posterior margin of the seventh sternum in the embryo and, in the nymphal or larval stages, the ends of these oviducts being derived from diverticula of the coelomic sacs of the seventh abdominal segment (Heymons, Wheeler, &c.). The mesodermal oviducts terminate thus in Ephemeroptera (Palmen, 1884; Heymons, 1896 *b*), Plecoptera (Heymons, 1899 *a*), Odonata (Heymons, 1896 *a*), Thysanura (Heymons, 1897), Orthoptera (Heymons, 1891, 1895 *a*; Wheeler, 1893), Dermaptera (Heymons, 1901; N.B. see under Forficula above, p. 48), Hymenoptera (Carrière and Bürger, 1897), Lepidoptera (Bessels, 1867; Jackson, 1889; Verson and Bisson, 1896; Zick, 1911), Homoptera (Heymons, 1899 *a*; George), Heteroptera (Heymons, 1899 *a*), Diptera (Weismann, 1866; Christophers, 1923; Christophers and Barraud, 1926), Coleoptera (Singh-Pruthi, 1924 *c*). It seems, therefore, that the mesodermal oviducts ending in the seventh segment must be regarded as fundamental in the insects. Now Palmen has shown that in the adult Ephemera this condition is retained, the paired mesodermal oviducts opening separately between the seventh and eighth sterna, no unpaired ectodermal oviducal portion being formed as in other insects. It appears to me, whether we hold the current conceptions of the law of biogenetics as stated by MacBride (1914, 1926) or agree with the restatement of this law by Garstang (1922), that the 'recapitulation' during ontogeny of an 'ephemerid' stage by other orders of insects as noted above admits of only one interpretation—that in insects in general (excluding the Collembola and Protura) the ancestral condition resembled that of some present-day Ephemera, double genital openings being present between the seventh and eighth sterna (strictly within the seventh intersternal membrane) leading directly into the paired mesodermal oviducts. Thus Palmen's main thesis of double genital apertures is admirably supported by ontogenetic evidence. Palmen, however, did not suggest the position of the paired genital openings between

the seventh and eighth sterna as an ancestral condition of insects, and indeed could not owing to the fragmentary ontogenetic evidence then available.

Granting the above, it seems reasonable to suppose as Palmen did that, following on the double gonopore stage, a short ectodermal common oviduct was acquired from the intersegmental membrane—an idea obviously involving no difficulty of explaining the transition between the double and single gonopore conditions. I must, however, definitely hypothecate an unpaired common oviduct invagination (see VII B (a) above, pp. 54 and 55), and further leave it open for this invagination to have been developed, not only from the seventh intersternal membrane but also from the extreme anterior portion of the eighth sternum which attaches to this membrane, i. e. in general from the immediate vicinity of the transverse limit between sterna 7 and 8. This hypothesis derives substantial support from the fact that in some admittedly primitive groups, e. g. Blattidae, Mantidae, Dermaptera (see VII B (b) above, pp. 55–57), Isoptera (Holmgren, 1909), the gonopore is located between the seventh and eighth sterna, a short unpaired common oviduct being developed from the seventh intersternal region as has been shown in *Blattella* and *Forficula*. A difficulty may appear to exist in that the gonopore in the ancient *Thysanura* (sens. strict.) is described as opening on the eighth sternum at the base of the ovipositor lobes (Meinert, 1871; Grassi, 1888), but this is easily met. From my studies on the Machilid *Petrobius carpenteri* Bagnall, I can state that in this form an unpaired common oviduct invagination arises in young stages between the seventh and eighth sterna; this condition is retained throughout development up to the adult when the short oviduct runs out into a short open groove on the anterior region of the eighth. Oudemans' description (1888) of the adult of *Petrobius maritimus* is substantially the same, so that the Machilidae give their weighty support to the hypothesis under discussion. It remains, however, for ontogeny to produce the conclusive evidence, viz. that in many groups the posteriorly placed gonopore is a secondary acquisition. It has been shown

that in *Locustidae* and *Tettigoniidae* the common oviduct originates as an invagination between the seventh and eighth sterna, and that it is later secondarily extended posteriorly, the gonopore being located on the inner reflexed surface of the eighth sternum. Striking support is further available in George's interesting discovery that in *Homoptera* the common oviduct invagination originates in young nymphs between sterna 7 and 8, and that it comes to open on the posterior part of the eighth in the adult by a secondary backward extension. A similar invagination between the seventh and eighth sterna probably exists in *Lepidoptera* (Jackson). Jackson has also shown that the common oviduct is secondarily extended posteriorly right on to the ninth sternum, the earlier phylogenetic phases being recapitulated during development.

The above considerations seem to point clearly to the acquisition of a common oviduct opening by a single gonopore between the seventh and eighth sterna as an early departure from the ancestral double genital aperture condition still retained by the *Ephemera*, a group which, notwithstanding high specialization in some respects, undoubtedly possesses many phylogenetically ancient features.

In view of the apparently universal recapitulation in the insects of the double genital aperture condition on the seventh sternum (see p. 59), it seems reasonable to predict that when the gap in our knowledge of the genital ducts in the different insect Orders has been filled up, an ontogenetic recapitulation of a common oviducal invagination between the seventh and eighth sterna will be found to exist in many other groups. In some, critical studies will no doubt also show that errors have been made in establishing the segmental position of the gonopore (cf. *Blattidae*, *Mantidae*, *Machilidae*). Two groups, however, in which the development of the genital ducts has been followed, deserve notice, as no mention of an invagination between the seventh and eighth sterna is made. Christophers (1923) in the *Mosquito* and Christophers and Barraud (1926) in *Phlebotomus* (*Diptera*), and Singh-Pruthi (1924 c) in *Tenebrio* (*Coleoptera*) describe the common oviduct as

originating as an invagination from the eighth sternum. Possibly these highly specialized groups do not recapitulate the invagination between the seventh and eighth sterna so clearly as do the Orthoptera and Homoptera. Or, alternatively, these insects departed from the double gonopore condition between the seventh and eighth sterna—recapitulated by these groups in the embryo and in the larva—by polyphyletically acquiring a common oviduct with the gonopore on the eighth sternum instead of between the seventh and eighth sterna.

Whether the common oviduct and single gonopore was acquired once only or more than once in the course of evolution is difficult to decide at present. There are, however, indications of a polyphyletic acquisition of this condition in the small but significant differences in place of origin of the common oviduct invagination between the seventh and eighth sterna (cf. its origin in Blattidae, Forficulidae, Locustidae, and in Homoptera as described by George). The origin of the common oviduct in Diptera (Christophers, Christophers and Barraud) from the median portion of the eighth sternum as well as the essentially similar finding of Singh-Pruthi in the Coleoptera can further be taken to favour this view. The backward extension of the common oviduct from the seventh sternum definitely seems to have taken place polyphyletically. Compare, for instance, its manner of extension in Locustidae with that described for Homoptera and also the final position of the gonopore in these groups. In Homoptera the gonopore is ultimately located dorsal of (posterior to) the anterior ovipositor lobes (George), in Locustidae ventral and anterior to these structures. Compare also the manner of extension of the common oviduct from the eighth to the ninth as described by Jackson in Lepidoptera and by Singh-Pruthi in Coleoptera (see II (b), pp. 29 and 31).

(d) The Accessory Genital Organs.

The occurrence of accessory genital organs in insects such as the spermatheca, various accessory glands, genital sacculae, &c., is well known. But apart from the work of Berlese (1909,

see under II (b), p. 30) no attempts to elucidate the morphology of these organs in insects in general have been made, as far as I know. My observations on the development of these structures in the Orthoptera together with a consideration of the comparative anatomical and ontogenetic evidence presented by other insects permit of important general deductions: (1) that the presence of two intrasegmental hypodermal invaginations from the eighth and ninth sterna must be postulated in the ancestors of those insects commonly grouped together as the Orthoptera, and (2) that this is a phylogenetic ancient condition, ancestral for insects in general.

(1) Orthoptera and Dermaptera.

Locustidae.—In *Locustana* and *Colemania* it has been shown above that two serially homologous, median unpaired hypodermal invaginations originate in the earliest nymphal instar on the eighth and ninth sterna from grooves between the bases of the anterior and lateral ovipositor lobes respectively. Later, when the inner ovipositor lobes are differentiated from the mesal margins of the lateral ovipositor lobes, the invagination on the ninth sternum opens between these structures. The invagination from the eighth sternum gives rise in the adult to the organ functioning as the spermatheca; the one on the ninth remains a short tubular organ, apparently functionless. As an entirely homologous invagination on the ninth sternum in *Blattella* (and other *Blattidae*) gives rise to accessory glands, the colleterial glands, it seems justifiable to regard this organ in *Locustidae* as vestigial, a remnant of a former accessory gland. This interpretation derives further support from the occurrence of such glands from the ninth sternum in other Orthoptera and orthopteroid groups, e. g. *Mantidae*, *Phasmidae*, *Isoptera*.

Tettigoniidae and *Gryllidae*.—As in the *Locustidae* only the invagination from the eighth sternum between the anterior ovipositor lobes is functional, and also as a spermatheca. Sections of gryllid nymphs (*Gryllus domesticus*

L.) show a corresponding vestigial accessory genital invagination on the ninth, between the inner ovipositor lobes.

Phasmidae.—In this group both accessory genital invaginations are present and functional, the one on the eighth sternum as a spermatheca (dissections of *Dixippus* and other Phasmids, sections of nymphs of *Dixippus*), the one on the ninth as a paired accessory gland with a common opening (cf. *Blattella*). In *Diapheromera* there is a paired spermatheca, according to Marshall and Severin (1906).

Blattidae and Mantidae.—In *Blattella*, as in *Locustana* and *Colemania*, two accessory genital invaginations are present in early nymphs on the eighth and ninth sterna between the anterior and lateral ovipositor lobes respectively; the one on the ninth likewise becomes located between the inner ovipositor lobes when they are differentiated. The invagination from the ninth sternum gives rise to the paired accessory gland (colleterial glands). The one from the eighth sternum, the homologue of the spermathecal invagination of *Locustana* and *Colemania*, atrophies in the late nymphal stages. There can be no doubt that this invagination was once functional as it persists in other Blattids, e.g. *Periplaneta* and *Blatta*, as a functional spermatheca (and spermathecal gland), as I have found by examining these forms and as is abundantly clear from the literature (Vogel, &c.). Its abortion in *Blattella* is a secondary feature and a peculiarity of this form, whose four spermathecae from the seventh intersegmental (intersegmental) membrane is aberrant (see also (e) below, pp. 67-71) and possibly due to a specialized mode of copulation.

Dermoptera.—In *Forficula* the invagination from the eighth functions in the adult as the spermatheca. It is interesting that it is in this form practically intersegmental, being located between sterna 8 and 9. This position is undoubtedly secondary if with Handlirsch (1908, 1918-25) and others we believe in an Orthopteran ancestral stock for the Dermoptera, and is almost certainly associated with the secondary loss of the ovipositor lobes and the reduction of the eighth sternum. No invagination is indicated during ontogeny or in the adult from

the ninth sternum, the tendency towards its atrophy manifested in Locustidae, Tettigoniidae, and Gryllidae being here carried to completion even ontogenetically.

From the facts presented above there is no difficulty of postulating as an ancestral condition in Orthoptera, accessory genital invaginations from the eighth and ninth sterna primitively developed in intimate association with the ovipositor lobes.

(2) In Insects in General.

A detailed morphological analysis of the accessory genital organs in all insects can as yet not be undertaken profitably owing to several difficulties. Chief amongst these is the lack of ontogenetic and especially comparative ontogenetic studies. Anatomical evidence alone cannot be relied upon owing to the great modifications of the genital segments and their invaginations and the consequent difficulty of correctly assigning the latter segmentally. Also, as has been illustrated above in the Orthoptera, in some forms one or the other of the invaginations may only be recapitulated during ontogeny, while sometimes, e.g. in *Forficula*, an invagination may lose its intrasegmental position secondarily. Moreover, complications arise when these independent invaginations secondarily come to open into the common oviduct (Lepidoptera, Jackson) or by an approximation of parts into a common vestibule (Diptera, Christophers). From the available data, however, there is little difficulty in recognizing the widespread existence of accessory genital invaginations from the eighth and ninth sterna, although in some members of an Order or lower group, one or the other of the invaginations or, exceptionally, both may be missing.

Genital invaginations have been found to arise during development from the eighth and ninth sterna in Lepidoptera (Jackson, Verson and Bisson), Diptera (Christophers, Christophers and Barraud), Coleoptera (Singh-Pruthi). In the Homopteron studied by George only the one from the ninth is developed. Comparative anatomical evidence shows accessory genital invaginations apparently from the eighth and ninth

sterna in Heteroptera (Ludwig, 1926), Thysanoptera (Klocke, 1926), Isoptera (Holmgren, 1909; Imms, 1919), Trichoptera (Stitz, 1904), Neuroptera (Stitz, 1909), Mecoptera (Stitz, 1908), Mallophaga (Strindberg), Hymenoptera (Seurat, 1899). In Plecoptera (Klapalek, 1896) and most Thysanura (Grassi, 1888; Oudemans, 1888; Willem, 1900) apparently only the invagination from the eighth is present. In some forms, however, e.g. Lepismids, an accessory gland (sebific) belonging to the ninth sternum is present (Grassi, loc. cit.).

From the general occurrence of accessory genital invaginations on the eighth and ninth sterna it seems plausible to suggest this as an ancestral condition of all insects, the primitive position of these invaginations being intrasegmental and in close association with the ovipositor lobes, as in Orthoptera. Phylogenetic specialization has shown itself in several ways. First by a loss or partial atrophy of one or the other of these invaginations, or of both, e.g. vestigial invagination from the ninth sternum in Locustidae, Tettigoniidae, Gryllidae, and its complete loss in Forficulidae, loss of the invagination from the eighth sternum in the adult in Homoptera (George) and in some Blattids, e.g. *Blattella*, where the invagination is, however, recapitulated during ontogeny. Further, in a complexity of structure, e.g. branching of accessory glands in Blattids, Mantids, and other insects, development of accessory spermathecal glands from the invagination from the eighth in Locustids, &c. Lastly, in functional specialization, best seen in the case of the invagination from the ninth sternum (e.g. into colleterial and sebific glands, poison glands (Hymenoptera, &c.). In addition to these primary accessory genital invaginations complementary accessory genital invaginations of a secondary nature seem to have been developed in Orders, families, and smaller groups, e.g. various genital sacculae, copulatory pouches, spermathecae of some Blattids (*Blattella*).

The question whether these accessory genital invaginations were originally paired or unpaired is not easy to answer. Evidence exists in favour of each condition. In Lepidoptera Jackson found the accessory glands from the ninth sternum to have

a paired origin, and in *Diptera* Christophers and Barraud are likewise inclined to so consider them. In *Blattella* (see above) the unpaired rudiment of the later paired accessory gland from the ninth sternum becomes double so soon that it is difficult to say whether this structure was not perhaps originally double. In other cases again the accessory genital invaginations are unpaired, e.g. in *Locustana*, *Colemania*, *Forficula* (see above); and *Tenebrio* (*Coleoptera*, Singh-Pruthi). In the *Machilid* *Petrobius* I have found the accessory invaginations from the eighth sternum to be definitely paired in origin and to develop from the bases of the coxites, suggesting a derivation from coxal glands. Pending further study on *Thysanuran* forms, I prefer to leave this question open.

One further point deserves consideration. Berlese and George evidently consider the invaginations from the eighth and ninth sterna to be serially homologous with the common oviduct invagination from the seventh sternum. From the accounts given above of the development and primitive intrasegmental position between the bases of the gonopods of these invaginations, and in some cases their paired origin, a serial homology with the unpaired common oviduct invagination seems to me to be precluded.

(e) The Spermatheca.

In the older literature (Dufour, 1834, 1841; Balbiani, 1870, 1872; Witlaczil, 1884; Schneider, 1883, 1885; Fenard, 1897) the spermatheca is generally described as a dorsal appendage of, and originating from, the 'vagina' (common oviduct). Later workers, recognizing that the genital cavity is morphologically distinct from the common oviduct, have found that in many insects the spermathecal invagination opens into the genital cavity independently of the oviduct (Haase, 1889; Bordas, 1909; Stitz, 1904; Holmgren, 1909; Vogel, 1925, &c.). In *Diptera* Christophers proved this ontogenetically by showing that the spermathecal invagination originates independently of the common oviduct invagination, and that later by an

approximation the two come to open in a secondarily formed genital atrium. Berlese (1909) concluded that the spermatheca is not always homologous morphologically—an idea which Leydig (1867) had already entertained. Recently George has subscribed to this view and with Berlese recognizes that the spermatheca may be developed from the seventh, eighth, or ninth sternites. No attempt, however, is made to elucidate the phylogeny of the organ. This is done by Singh-Pruthi, who, finding that the spermathecal rudiment in *Tenebrio* (Coleoptera) originates as an invagination from the ninth sternite, postulates this as the primitive condition for insects.

My observations above entirely support the conclusion of Leydig, Berlese, and George that the spermatheca is not always homologous. In Locustidae (*Locustana* and *Colemania*), Gryllidae (*Gryllus*), Phasmidae (*Dixippus*), Forficulidae (*Forficula*), the spermatheca originates as an invagination from the eighth sternum independently of the common oviduct invagination. It opens in this position and manner also in the adult. Examination of adults of Tettigoniidae (*Phasgonura*, &c.), Mantidae (*Mantis*), and Blattidae (*Blatta*, *Periplaneta*) show the spermatheca to open similarly. In *Blattella* (Blattidae) an anomalous condition exists in that four spermathecae are present which originate from, and open on, the seventh intersternal membrane. *Blattella*, then, secondarily departs from the common orthopteran condition where the spermatheca originates and opens between the bases of the anterior ovipositor lobes on the eighth sternum as a comparison with other Blattids shows, and as is indicated by its recapitulation during development of the spermathecal invagination from the eighth; its spermathecae in no way correspond morphologically with those of the other Orthoptera. Further cases of the morphological diversity of the spermatheca may be cited, for instance its origin in Homoptera as a diverticulum of the common oviduct invagination from the seventh sternum (George) and in Coleoptera as an invagination from the ninth (Singh-Pruthi).

It is evident by now that the term 'spermatheca' has a

'functional' rather than 'morphological' significance. Yet it seems to me that it may be connoted morphologically as well. It has been shown above (VII (d), pp. 62-67) that accessory genital invaginations from the eighth and ninth sterna, between the ovipositor lobes, represent an ancestral condition for Orthoptera and almost certainly for insects in general. Now it appears to be significant that a spermathecal function can be related with remarkable frequency to the accessory genital invagination(s) on the eighth sternum. And further that in the admittedly primitive and generalized insects this association is almost universally true. The conclusion is therefore not unwarranted that the spermathecal function was primitively allocated to an invagination or invaginations from the eighth sternum in insects in general. To consider the lower orders first. It has been shown above that the spermathecal function is relegated to such an invagination(s) in all groups of the Orthoptera and also in the Dermaptera. In Blattidae the one exception noted, *Blattella*, proves the rule as the common condition is recapitulated during ontogeny. In other 'orthopteroid' insects the spermatheca is likewise an invagination from the eighth sternum, in Plecoptera (Klapalek, 1896), Odonata (diverticulum from eighth invagination, George), and almost certainly in Isoptera, as the figures and descriptions of Holmgren (1909) and Imms (1919) show by comparison with the related Blattids, although these authors do not agree in numerically designating the genital segments; in the Thysanura (*Machilidae*, Oudemans, 1888; *Lepismidae*, *Campodeidae*, *Japygidae*, Grassi, 1888) as well, although Grassi differs from Oudemans in considering the general cavity as a 'vagina' and thus describes the spermatheca as a dorsal diverticulum of the latter. In *Petrobius* I have found that the two spermathecae originate separately and independently of the common oviduct at the bases of the coxites.

Considering other Orders, the spermathecal invagination belongs to the eighth sternum in the Heteroptera (Ludwig, 1926) and apparently also in the Thysanoptera (Klocke, 1926). In Diptera Christophers (1923) and Christophers and Barraud

(1926) describe the spermatheca as originating from the eighth sternum or possibly between the eighth and ninth (cf. the secondary intersegmental position of this invagination in *Forficula*). In *Lepidoptera* Jackson (1890) and Verson and Bisson (1896) describe the 'bursa copulatrix' as developing from the eighth sternum. The single genital opening of *Micropterygidae* is evidently secondary, as Tillyard (1922) has discovered in the pupa of *Sabatinca* what is apparently a vestigial second genital opening, and such an opening has been noted on the eighth sternum in the pupa of *Mnemonica* by Mosher (1916).

It remains to consider the opposing view of Singh-Pruthi, who is apparently the only author who has dealt with the primitive position of the spermatheca. Singh-Pruthi's hypothesis that the spermatheca is primitively developed as an unpaired invagination from the ninth sternum can readily be shown to be untenable as it is not only open to grave objections but rests largely on incorrect data. An important objection is the observation of Jackson (loc. cit.) that the 'bursa copulatrix' in *Lepidoptera* definitely develops as an invagination from the eighth sternum. Singh-Pruthi, recognizing this, devotes fully a page to discrediting Jackson's interpretation asking (loc. cit., p. 880), 'Why should the spermathecal opening, primitively behind the ninth sternite in all insects (*sic*!), be behind the eighth in *Lepidoptera*?' and further stating that the anterior opening should be the 'uterus' opening, the posterior the spermathecal. That the anterior genital opening in *Lepidoptera* is the copulatory opening is such a well-established fact (see Poulton, 1890; Petersen, 1900; Stitz, 1901; Berlese, 1909; Deegener, 1913-21; Imms, 1924, &c.) that it is difficult to understand Singh-Pruthi's denial of this. His further statement that the spermatheca opens primitively behind the ninth sternum in all insects is entirely unfounded, as is apparent from the facts marshalled above and it certainly does not open on the ninth sternum in *Blattidae* and *Acridiidae* (*Locustidae*) as stated by this author (loc. cit., p. 879). Far from representing a primitive or even common condition, the spermathecal invagina-

tion from the ninth sternum in *Tenebrio* appears to be exceptional and specialized.

(f) Concluding Remarks.

The ovipositor lobes on the eighth and ninth abdominal sterna appear in a different light now that the primitive position of the gonopores has been established between the seventh and eighth sterna. These structures, which are undoubtedly derived from former appendages (see II (a), pp. 27-29), probably first subserved a copulatory and fertilizing function, rather than an ovipositing one—a plausible suggestion considering that sperm was primitively passed to the female probably by means of portable spermatophores, and the probable allocation primitively of a spermathecal function to the accessory genital invagination(s) between the bases of the appendages of the eighth sternum.

In conclusion, two general tendencies in the specialization of the oviducal system remain to be noted. Berlese (1909) observed that in general in the higher groups the gonopore tends to be more posteriorly placed. This is given an ontogenetic basis above and a phylogenetic significance. A tendency is further evident for an ever-increasing exploitation of the ectoderm—witnessed in the posterior extension of the common oviduct—within the higher groups a possible encroachment into the mesodermal oviducts and an eventual replacement of these—a tendency already noted by Deegener (1913-21). Brüel (1899) in *Calliphora* (Diptera) and Korschelt (1924) in *Dytiscus* (Coleoptera) found the paired oviducts to be definitely lined with ectoderm. As embryological evidence shows that these structures are definitely of mesodermal origin, this anomaly seems to me to depend on a secondary replacement of the mesodermal epithelium by hypoderm during development, broadly analogous perhaps with the replacement of the mid-gut epithelium by ectoderm in higher insects (see Mansour, 1927). This point, however, must be settled ontogenetically, assuming that Brüel and Korschelt are correct in their findings.

In the Orthoptera studied by me the mesodermal epithelium of the paired oviducts is certainly not replaced by ectodermal epithelium.

VIII. SUMMARY AND CONCLUSIONS.

1. The anterior ovipositor lobes (ventral valves) of Orthoptera are serially homologous with the lateral ovipositor lobes (dorsal valves) and represent gonocoxites from which no telopodites, corresponding to the inner valves, are differentiated.

2. The common oviduct originates as an unpaired ectodermal (hypodermal) invagination, in *Blattella* from the seventh intersternal membrane, in *Locustana*, *Colemania*, and *Forficula* between the seventh and eighth sterna, ending blindly, internally, between the ends of the mesodermal oviducts lying near the posterior margin of the seventh sternum.

3. In *Blattella* and *Forficula* the early condition is retained up to the adult stage; in *Locustana* and *Colemania* the common oviduct is secondarily extended posteriorly along the eighth sternum in the first instar, the gonopore coming to open in later instars, and in the adult on the inner reflexed surface of the eighth sternum.

4. Grooves are present in the early stages of *Locustana*, *Colemania*, and *Blattella*, between the bases of the ovipositor lobes on the eighth and ninth sterna, which become tubular anteriorly, giving rise each to an accessory genital invagination. The invagination from the eighth gives rise to the spermatheca in *Locustana* and *Colemania*; in *Blattella* it atrophies in late nymphal stages. The invagination from the ninth gives rise to the paired colleterial gland in *Blattella*; in *Locustana* and *Colemania* it develops slowly and gives rise to a vestigial accessory genital invagination. In *Forficula* no ovipositor lobes are present, and the invagination from the eighth arises practically intersegmentally between sterna 8 and 9. It also gives rise to the spermatheca in the adult. No accessory genital invagination is developed from the ninth sternum.

5. In *Blattella* four spermathecae are present. They

originate as two pairs of separate hypodermal invaginations from the seventh intersternal membrane, morphologically posterior to the common oviduct invagination.

6. The primitive position of the single gonopore in the Orthoptera and Dermaptera is between sterna 7 and 8, its position in present-day Locustidae, Tettigoniidae, Gryllidae on the eighth sternum being secondary.

7. The spermathecal function was ancestrally associated with the accessory genital invagination from the eighth sternum in the Orthoptera. The four spermathecae of *Blattella* represent secondarily developed structures which do not correspond to the spermathecae of other Blattids, e.g. *Periplaneta* and other Orthoptera.

8. There is no evidence that part of the common oviduct is of mesodermal derivation or that it has a paired origin in the Orthoptera and in insects in general.

9. A hypothetical ancestral condition of the oviducal system and accessory organs is deduced for insects in general. The paired mesodermal oviducts opened separately within the seventh intersternal membrane, two gonopores being present. Accessory genital invaginations, possibly paired, were present on the eighth and ninth sterna at the bases of the gonopods, a spermathecal function probably being associated with the invagination(s) on the eighth sternum. Phylogenetic specialization consisted in an early acquisition of an ectodermal common oviduct and single gonopore between the seventh and eighth sterna, possibly polyphyletic, and a later extension posteriorly of the common oviduct and single gonopore on to the eighth and on to the ninth sterna, this posterior extension having definitely taken place polyphyletically.

IX. ACKNOWLEDGEMENTS.

My sincere thanks are due to Professor F. Balfour-Browne for supervising this work and for the very keen interest he has taken in its progress. I am further greatly indebted to Dr. J. W. Munro for his interest in my work and for the many favours I have received at his hands. To Dr. C. J. George I am very

grateful for his unfailing willingness to discuss with me the problems of the present research, for generously supplying me with the *Colemania* material, and lastly for allowing me to refer to the manuscript of his then unpublished paper.

APPENDIX.

After the present paper had been completed, my notice was drawn to an important paper by Zander in which Löscher deals with the post-embryonic development of the oviducal system and accessory organs in the queen of the honey-bee. As this work (E. Zander, 1916, 'Die Ausbildung des Geschlechtes bei der Honigbiene (*Apis mellifica* L.). III. Die postembryonale Entwicklung des Geschlechtsapparates der Bienenkönigin', 'Zeitschr. f. Angew. Entom.', 3) seems to have escaped the notice of all recent workers on this subject (e.g. Singh-Pruthi, &c.) its main contents may be briefly considered. Löscher found the mesodermal oviducts to terminate in the seventh segment in the earliest larvae. The common oviduct originates from the posterior region of the seventh sternum and becomes secondarily extended on to the eighth. The spermatheca originates entirely independently of the common oviduct from a groove between the bases of the anterior gonapophyses ('lancets') as a paired invagination on the eighth sternum. The large poison gland (acid gland) originates from a similar groove between the bases of the inner gonapophyses on the ninth sternum ('sheath of sting') as an unpaired invagination. These findings in the Hymenoptera strikingly support my main conclusions. In the adult the paired oviducts are ectodermal, and Löscher shows that a gradual replacement of the mesodermal epithelium takes place during development—thus providing a basis of observed fact to my supposition given in VII (e).

BIBLIOGRAPHY.

- Balbiani, E. G. (1870).—"Mémoire sur la génération des Aphides", 'Ann. Sc. nat. (Zool.)', 14.
- Bekker, E. (1925).—"The structure and development of the external genitalia of Thysanura and Hymenoptera" (in Russian), 'Trud. nauchno-issledovat. Inst. Zool. Moscow', 1.
- Berlese, A. (1882).—"Ricerche sugli organi genitali degli Ortotteri", 'Atti della R. Accad. d. Lincei', 3a ser., 11.
- (1909).—"Gli Insetti, loro organizzazione, sviluppo, abitudini e rapporti coll' uomo", 1. Milan.
- Bessels, E. (1867).—"Studien über die Entwicklung der Sexualdrüsen bei den Lepidopteren", 'Zeitschr. f. wiss. Zool.', 17.
- Bordas, L. (1894).—"Appareil glandulaire des Hyménoptères", 'Ann. Sc. nat. (Zool.)', 19.
- (1909).—"Recherches anatomiques, histologiques et physiologiques sur les organes appendiculaires de l'appareil reproducteur femelle des Blattes (*P. orientalis*)", *ibid.*, ser. 9, 9.
- Börner, C. (1904).—"Zur Klärung der Beingliederung der Ateloceraten", 'Zool. Anz.', 27.
- Brüel, L. (1897).—"Anatomie und Entwicklungsgeschichte der Geschlechtsausführwege samt Annexen von *Calliphora erythrocephala*", 'Zool. Jahrb., Abt. Anat. u. Ontog.', 10.
- Bugnion, E., et N. Popoff (1912).—"Anatomie de la Reine et du Roi-Termite", 'Mém. Soc. Zool. France', 25.
- (1923).—"Mantes et Empuses", 'Mém. Soc. Vaud. Sc. nat., Lausanne', 1.
- Carrière, J., und O. Bürger (1897).—"Die Entwicklungsgeschichte der Mauerbiene (*Chalicodoma muraria* Fabr.) im Ei", 'Nova Acta Acad. Leop. Carol.', 69.
- Cholodkowsky, N. (1885).—"Über den Geschlechtsapparat von *Nematois metallicus*", 'Zeitschr. f. wiss. Zool.', 42.
- (1891).—"Die Embryonalentwicklung von *Phyllodromia* (*Blatta*) *germanica*", 'Mém. Acad. Imp. d. Sc. St. Pétersbourg', 38.
- Chopard, L. (1920).—"Recherches sur la conformation et le développement des derniers segments abdominaux chez les Orthoptères." Thèse, Rennes. Imprimerie Oberthur.
- Christophers, S. R. (1923).—"The structure and development of the female genital organs and hypopygium of the Mosquito", 'Ind. Journ. Med. Res.', 10.
- Christophers, S. R., and P. J. Barraud (1926).—"The development of the male and female hypopygium of *Phlebotomus*", *ibid.*, 13.
- Claypole, Agnes (1898).—"The embryology and oogenesis of *Anurida maritima* Guér.", 'Journ. Morph.', 14.

- Crampton, G. C. (1917).—"A phylogenetic study of the terminal abdominal segments and appendages in some female apterygotan and lower pterygotan insects", 'Journ. New York Entom. Soc.', 25.
- (1919).—"Notes on the phylogeny of the Orthoptera", 'Entom. News', 30.
- (1921).—"A comparison of the terminal abdominal structures of insects and crustaceans", *ibid.*, 32.
- (1923).—"A comparison of the terminal abdominal structures of the female of a termite (*Masotermes*) with those of the roach *Periplaneta*", 'Bull. Brooklyn Entom. Soc.', 18.
- (1925).—"The external anatomy of the head and abdomen of the roach, *Periplaneta americana*", 'Psyche', 32.
- Deegener, P. (1913-21).—"Geschlechtsorgane" in Schröder, 'Handbuch der Entomologie', 1.
- Denny, A. (1893).—"On the development of the 'Ovipositor' in the cockroach", 'Rept. 63 Brit. Assoc. Adv. Sci. (Lond.)'.
- Dewitz, H. (1875).—"Ueber Bau und Entwicklung des Stachels und der Legescheide einiger Hymenoptera und der grünen Heuschrecke", 'Zeitschr. f. wiss. Zool.', 25.
- Dufour, L. (1841).—"Recherches anatomiques et physiologiques sur les Orthoptères, les Hyménoptères et les Névroptères", 'Mém. d. Savants étrang. Acad. Roy. Inst. France', 7.
- Escherich, K. (1905).—"Das System der Lepismatiden", 'Zoologica', 18.
- Ezhikov, I. (1925).—"On the morphology and importance of the ovipositor and sting of Insects" (in Russian), 'Trud. nauchno-issledovat. Inst. Zool. Moscow', 1.
- Fenard, A. (1897).—"Recherches sur les organes complémentaires internes de l'appareil génital des Orthoptères", 'Bull. Sc. France et Belg.', 29.
- Ganin, M. (1869).—"Beiträge zur Erkenntnis der Entwicklungsgeschichte bei den Insecten", 'Zeitschr. f. wiss. Zool.', 19.
- Garstang, W. (1921).—"The Theory of Recapitulation—a critical restatement of the Biogenetic Law", 'Linn. Soc. Journ. (Zool.)', 35.
- Grassi, B. (1888).—"Anatomia comparata dei Tisanuri e considerazioni generali sull'organizzazione degli Insetti", 'Atti R. Accad. d. Lincei d. Roma, Mem. Cl. sci. fis., etc.', ser. 4, 4.
- Haase, E. (1889 a).—"Die Abdominalanhänge der Insekten mit Berücksichtigung der Myriapoden", 'Morph. Jahrb.', 15.
- (1889 b).—"Die Zusammensetzung des Körpers der Schaben", 'Sitzungsber. d. Ges. Naturf. Freunde, Berlin, 1889'.
- Handlirsch, A. (1904).—"Zur Systematik der Hexapoden", 'Zool. Anz.', 27.
- (1908).—"Die fossilen Insekten und die Phylogenie der rezenten Formen." Leipzig.

- Handlirsch, A. (1913-25).—"Phylogenie oder Stammesgeschichte" in Schröder, 'Handbuch der Entomologie', 3.
- Henneguy, L. F. (1904).—"Les Insectes." Paris.
- Herold, M. J. D. (1815).—"Entwicklungsgeschichte der Schmetterlinge anatomisch und physiologisch bearbeitet." Cassel und Marburg.
- Heymons, R. (1891).—"Die Entwicklung der weiblichen Geschlechtsorgane von *Phyllodromia* (*Blatta* L.) *germanica*", 'Zeitschr. f. wiss. Zool.', 53.
- (1895 a).—"Die Embryonalentwicklung von *Dermapteren* und *Orthopteren*." Jena.
- (1895 b).—"Ueber die abdominalen Körperanhänge der Insekten", 'Biol. Centralbl.', 24.
- (1896 a).—"Zur Morphologie der Abdominalanhänge bei den Insekten", 'Morph. Jahrb.', 24.
- (1896 b).—"Grundzüge der Entwicklung und des Körperbaues von *Odonaten* und *Ephemeren*", 'Anhang zu Abh. K. Akad. d. Wiss.' Berlin, 1896.
- (1897).—"Entwicklungsgeschichtliche Untersuchungen an *Lepisma saccharina* L.", 'Zeitschr. f. wiss. Zool.', 62.
- (1899 a).—"Beiträge zur Morphologie und Entwicklungsgeschichte der *Rhynchoten*", 'Nova Acta Acad. Leop. Carol.', 74.
- (1899 b).—"Der morphologische Bau des Insektenabdomens", 'Zool. Centralbl.', 6.
- (1901).—"Die Entwicklungsgeschichte der *Scolopender*", 'Zoologica', 13.
- Hirschler, Jan. (1924-7).—"Embryogenese der Insekten" in Schröder, 'Handbuch der Entomologie', 1.
- Holmgren, N. (1909).—"Termitenstudien. I. Anatomische Untersuchungen", 'Kungl. Svenska Vetenskapsakad. Handlingar', 44.
- Huxley, T. H. (1877).—"The Anatomy of Invertebrated Animals." London.
- Imms, A. D. (1906).—"Anurida", 'Liverpool Mar. Biol. Comm. Memoirs', 13.
- (1919).—"On the structure and biology of *Archotermopsis*, together with descriptions of new species of intestinal Protozoa and general observations on the *Isoptera*", 'Phil. Trans. Roy. Soc.', Ser. B, 209.
- (1925).—"A General Text-book of Entomology." London.
- Jackson, W. Hatchett (1890).—"Studies in the morphology of the *Lepidoptera*, I", 'Trans. Linn. Soc. London', 2nd ser., Zool., 5.
- Klapalek, Fr. (1896).—"Über die Geschlechtsteile der *Plecopteren*, mit besonderer Rücksicht auf die Morphologie der Genitalanhänge", 'Sitzungsber. K. Akad. Wiss. Wien, Math.-Naturwiss. Cl.', 105.
- Klocke, F. (1926).—"Beiträge zur Anatomie und Histologie der *Thysanopteren*", 'Zeitschr. f. wiss. Zool.', 128.

- Kraepelin, C. (1873).—"Untersuchungen über den Bau, Mechanismus und Entwicklungsgeschichte des Stachels der bienenartigen Thiere", 'Zeitschr. f. wiss. Zool.', 23.
- Korschelt, E. (1924).—(Herausgegeben von.) 'Der Gelbrand—*Dytiscus marginalis*', 2. Leipzig.
- Korschelt, E., and K. Heider (1899).—"Text-book of the Embryology of Invertebrates", 3. London.
- Lacaze-Duthiers, F. J. H. (1849–53).—"Recherches sur l'armure génitale femelle des Insectes", 'Ann. d. Sc. nat. (Zool.)', 12. 14 (Hyménoptères), 17 (Orthoptères), 18 (Hémiptères), 19 (Neuroptères, Thysanoures, Coléoptères, Diptères, Lépidoptères, Aphaniptères en général).
- Leydig, Fr. (1867).—"Der Eierstock und die Samentasche der Insecten", 'Nova Acta Acad. Leop. Carol.', 33.
- Lowne, B. T. (1889).—"On the structure and development of the ovaries and their appendages in the Blow-fly (*Calliphora erythrocephala*)", 'Journ. Linn. Soc. London', 20.
- Ludwig, W. (1926).—"Untersuchungen über den Copulationsapparat der Baumwanzen", 'Zeitschr. f. Morph. u. Ökol. d. Tiere', 5.
- MacBride, E. W. (1914).—"Text-book of Embryology", 1. Invertebrata.
- (1926).—"The Recapitulation Theory", 'Science Progress', 22.
- Mansour, K. (1927).—"The development of the larval and adult mid-gut of *Calandra oryzae* (Linn.): the rice weevil", 'Quart. Journ. Micr. Sci.', 71.
- Marshall, Wm. S., und Henry Severin (1905).—"Über die Anatomie der Gespenstheuschrecke, *Diapheromera femorata* Say", 'Arch. f. Biontologie', 1.
- Mayer, P. (1876).—"Ueber Ontogenie und Phylogenie der Insekten", 'Jena. Zeitschr. f. Naturwiss.', 10.
- Meinert, F. (1863).—"Anatomia Forficularum", 'Anatomisk undersøgelse af de Danske Orentviste, I. Naturhist. Tidsskr. 3 Raek.', 3.
- (1871).—"Om Kjönnorganerne og Kjønnsstoffernes Udvikling hos *Machilis polypoda*", *ibid.*, 7.
- Miall, L. C., and A. Denny (1886).—"The structure and life-history of the Cockroach (*Periplaneta orientalis*).", London.
- Mosher, Edna (1916).—"A classification of the Lepidoptera based on the characters of the pupa", 'Bull. Illinois State Lab. Nat. Hist.', 12.
- Nusbaum, J. (1882).—"Zur Entwicklungsgeschichte der Ausführungsgänge der Sexualdrüsen bei den Insecten", 'Zool. Anz.', 5.
- (1884).—*Ibid.* (Polish, with résumé in German), 'Kosmos, Lemberg', 9.
- Oudemans, J. T. (1888).—"Beiträge zur Kenntnis der Thysanura und Collembola", 'Bijdr. tot Dierk.', 16. (Transl. of thesis published in Dutch, Amsterdam, 1877.)

- Packard, A. S. (1868).—"On the structure of the ovipositor and homologous parts in the male insect", 'Proc. Boston Soc. Nat. Hist.', 11.
- (1871).—"Embryological studies on *Diplax*, *Perithemis*, and the thysanourous genus *Isotoma*", 'Mems. Peabody Acad. Sc. Salem, Mass.', 1.
- (1898).—"A Text-book of Entomology." London.
- Palmen, J. A. (1883).—"Zur vergleichenden Anatomie der Ausführungsgänge der Sexualorgane bei den Insecten", 'Morph. Jahrb.', 9.
- (1884).—"Über paarige Ausführungsgänge der Geschlechtsorgane bei Insecten." Helsingfors.
- Petersen, W. (1900).—"Beiträge zur Morphologie der Lepidopteren", 'Mém. Acad. Imp. d. Sc. St.-Petersbourg', v, 9.
- Peytoureau, S. A. (1895).—"Contribution à l'étude de la morphologie de l'armure génitale des Insectes." Paris.
- Poulton, E. B. (1890).—"The External Morphology of the Lepidopterous pupa; its relation to that of other stages and to the origin and history of metamorphosis", 'Trans. Linn. Soc. London', 2nd ser., 5.
- Schimmer, F. (1901).—"Beitrag zu einer Monographie der Gryllodeengattung *Myrmecophila* Latr.", 'Zeitschr. f. wiss. Zool.', 93.
- Schneider, A. (1883-5).—"Über die Entwicklung der Geschlechtsorgane der Insecten." In Heft I u. III, 'Zool. Beiträge v. Anton Schneider', 1.
- Schröder, Chr. (1912-28).—(Herausgegeben von.) 'Handbuch der Entomologie,' Bd. I, 1912-28; Bd. III, 1913-25. Jena.
- Seidel (1924).—"Die embryonale Entwicklung der Geschlechtsorgane von *Pyrrhocoris apterus* L.", 'Zeitschr. f. Morph. u. Ökol. d. Tiere', 1.
- Seurat, L. G. (1899).—"Contributions à l'étude des Hyménoptères entomophages", 'Ann. d. Sc. nat. (Zool.)', ser. 8, 10.
- Sinety, R. de (1901).—"Recherches sur la biologie et l'anatomie des Phasmes", 'La Cellule', 19.
- Singh-Pruthi, Hem. (1924 a).—"The development of the male genitalia of Homoptera, with preliminary remarks on the nature of these organs in other insects", 'Quart. Journ. Micr. Sc.', 69.
- (1924 b).—"On the post-embryonic development and homologies of the male genital organs of *Tenebrio molitor* L. (Coleoptera)", 'Proc. Zool. Soc. London', 1924.
- (1924 c).—"On the development of the ovipositor and the efferent genital ducts of *Tenebrio molitor* L. (Coleoptera), with remarks on the comparison of the latter organs in the two sexes", *ibid*.
- Sokolow, A. J. (1926).—"Zur Frage der Spermatophorbefruchtung bei der Wanderheuschrecke (*Locusta migratoria* L.). Das Weibchen", 'Zeitschr. f. wiss. Zool.', 127.
- Stitz, H. (1901).—"Der Genitalapparat der Mikrolepidopteren. (2) Der weibliche Genitalapparat", 'Zool. Jahrb., Abt. Anat. u. Ontog.', 15.

- Stitz, H. (1904).—"Zur Kenntnis des Genitalapparats der Trichopteren", 'Zool. Jahrb., Abt. Anat. u. Ontog.', 20.
- (1908).—"Zur Kenntnis des Genitalapparats der Panorpaten", *ibid.*, 26.
- (1909).—"Zur Kenntnis des Genitalapparats der Neuropteren", *ibid.*, 27.
- Strindberg, H. (1916 a).—"Zur Entwicklungsgeschichte und Anatomie der Mallophagen", 'Zeitschr. f. wiss. Zool.', 115.
- (1916 b).—"Studien über die ectodermalen Teile der Geschlechtsorgane einiger Mallophagengattungen", 'Zool. Anz.', 48.
- (1918).—"Typstudien über die Geschlechtsorgane einiger Mallophagengattungen", 'Zeitschr. f. wiss. Zool.', 117.
- (1919).—"Die Geschlechtsorgane von *Ornithobius bucephalus* Gieb. und *Goniodes falcicornis* N.", 'Zool. Anz.', 50.
- Suckow, F. W. L. (1928).—"Geschlechtsorgane der Insecten", Heusinger's 'Zeitschr. f. organ. Physik', 2.
- Tillyard, R. J. (1923).—"On the larva and pupa of the genus *Sabatinca*", 'Trans. Entom. Soc. London', 1922.
- (1926).—"The Insects of Australia and New Zealand." Sydney.
- Uljanin, B. (1872).—"Die Entwicklung des Stachels der Arbeitsbiene" (in Russian). Communication by Kovalevsky in 'Zeitschr. f. wiss. Zool.', 22.
- Van der Weele, H. W. (1906).—"Morphologie und Entwicklung der Gonapophysen der Odonaten", 'Tijdschr. v. Entom.', 49.
- Verhoeff, C. (K. W.) (1895).—"Cerci und Styli der Tracheaten", 'Entom. Nachricht.' (F. Karsch), 21.
- (1896).—"Zur Morphologie der Segmentanhänge bei Insekten und Myriopoden", 'Zool. Anz.', 19.
- (1897).—"Bemerkungen über abdominale Körperanhänge bei Insekten und Myriopoden", *ibid.*, 20.
- (1898).—"Noch einige Worte über Segmentanhänge bei Insekten und Myriopoden", *ibid.*, 21.
- (1902).—"Zur vergleichenden Morphologie der Coxalorgane und Genitalanhänge der Tracheaten", *ibid.*, 26.
- Verson, E., und E. Bisson (1896).—"Die postembryonale Entwicklung der Ausführungsgänge und der Nebendrüsen beim weiblichen Geschlechtsapparat von *Bombyx mori*", 'Zeitschr. f. wiss. Zool.', 61.
- Vogel, R. (1925).—"Bemerkungen zum weiblichen Geschlechtsapparat der Küchenschabe *Periplaneta*", 'Zool. Anz.', 64.
- Walker, E. M. (1919).—"The terminal abdominal structures of Orthopteroid insects—a phylogenetic study. I. The terminal abdominal structures of the female", 'Ann. Entom. Soc. America', 12.
- (1922).—*Ibid.* "II. The terminal abdominal structures of the male", *ibid.*, 15.

- Weismann, A. (1864).—"Die nachembryonale Entwicklung der Musciden", 'Zeitschr. f. wiss. Zool.', 14.
- (1866).—"Die Metamorphose der *Corethra plumicornis*", *ibid.*, 16.
- Wheeler, W. M. (1889).—"The embryology of *Blatta germanica* and *Doryphora decemlineata*", 'Journ. Morph.', 3.
- (1893).—"A contribution to insect embryology", *ibid.*, 8.
- Wille, Joh. (1920).—"Biologie und Bekämpfung der deutschen Schabe", 'Beiheft 1 zu Zeitschr. f. angew. Entom.', 7.
- Willem, V. (1900).—"Recherches sur les Collembolés et les Thysanoures", 'Mém. d. Sav. étrang. Acad. R. Belg.', 58.
- Witlaczil, E. (1884).—"Entwicklungsgeschichte der Aphiden", 'Zeitschr. f. wiss. Zool.', 40.
- Zacher, Fr. (1911).—"Studien über das System der Protodermapteren", 'Zool. Jahrb., Abt. Syst.', 30.
- Zick, K. (1911).—"Beiträge zur Kenntnis der postembryonalen Entwicklungsgeschichte der Genitalorgane bei Lepidopteren", 'Zeitschr. f. wiss. Zool.', 98.

EXPLANATION OF PLATES 2-4.

All figures are from camera lucida drawings. The ventral portions only of the genital segments are shown in cross-section.

LIST OF COMMON ABBREVIATIONS.

acc inv, accessory genital invagination; *amp*, ampulla; *an*, anus; *a ovip*, anterior ovipositor lobe; *ap*, apodeme; *b-*, basal portion of; *ca*, 'boyau-calicial'; *coll gl*, colleterial gland; *c od*, common oviduct; *c od gr*, common oviduct groove; *c od inv*, common oviduct invagination; *d pch*, dorsal pouch; *e clx*, egg-calyx; *eg*, egg-guide; *fld(s)*, fold(s) of genital cavity floor; *fcps*, forceps; *gen cav*, genital cavity; *gl c*, tract of glandular cells; *gr*, groove; *go*, gonopore; *i ovip*, inner ovipositor lobe; *int*, intestine; *l fld*, lateral fold; *l ovip*, lateral ovipositor lobe; *l pch*, lateral pouch; *mes od*, mesodermal oviduct; *mu*, muscles; *nc*, nerve-cord; *ng*, nerve ganglion; *o coll gl*, opening of colleterial gland; *ov*, ovary; *pa*, paraproct; *pct*, pocket-like invagination; *p gl*, pygidial stink-gland of nymphs; *rec*, rectum; *r-*, rudiment of; *s*, sternum; *spm*, spermatheca; *spm duct*, spermathecal duct; *spm inv*, spermathecal invagination; *spm gr*, sperm-guiding groove; *spm po*, spermathecal pore; *st*, stylus; *subg pl*, subgenital plate; *sup an pl*, supra-anal plate; *T*, tergum; *tra*, trachea.

Roman figures refer to the number of the abdominal segments morphologically.

PLATE 2.

Figs. 1-6.—*Locustana*, early first instar nymph. Cross-sections from a series proceeding anteriorly from the caudal end of the abdomen.

Fig. 1.—Through base of lateral ovipositor lobe rudiments showing groove (*gr l*) between them. $\times 75$.

Fig. 2.—A few sections anteriorly showing groove becoming a tubular invagination, the rudiment of the accessory genital invagination (*acc inv 1*). $\times 350$.

Fig. 3.—Through base of anterior ovipositor lobe rudiments on eighth sternum showing their lateral attachment and the groove (*gr 2*) between them. $\times 75$.

Fig. 4.—A few sections anteriorly showing groove becoming tubular, the rudiment of the spermathecal invagination (*spm inv* or *acc inv 2*). $\times 350$.

Fig. 5.—Through anterior end of eighth segment near junction with seventh showing common oviduct invagination running out groove-like (*c od gr*) on its ventral surface. $\times 75$.

Fig. 6.—A few sections anteriorly showing incompletely tubular common oviduct invagination (*c od inv*). $\times 75$.

Fig. 7.—*Colemania*, early first instar nymph. Cross-section through posterior part of seventh segment showing common oviduct invagination (*c od inv*) ending between the ampullar ends (*amp*) of the paired mesodermal oviducts (*mes od*). $\times 145$.

Figs. 8-12.—*Colemania*, 14 mm. (second instar of *Locustana*).

Cross-sections from a series proceeding cephalad. $\times 125$.

Fig. 8.—Through base of lateral ovipositor lobes (*l ovip*) showing inner ovipositor lobes (*i ovip*) arising mesally as outgrowths from them and now bounding the groove (*gr 1*).

Fig. 9.—Through base of anterior ovipositor lobes (*a ovip*) showing eighth sternum overgrowing their bases. Note absence of outgrowths on their mesal margins.

Fig. 10.—A few sections anteriorly showing spermathecal invagination (*spm inv*) and the formation of a genital cavity (*gen cav*) by the overgrowing eighth sternum.

Fig. 11.—A few sections more anteriorly showing common oviduct (*c od*) opening into rudimentary genital cavity (*gen cav*).

Fig. 12.—More anteriorly still showing genital cavity prolonged anteriorly above common oviduct (*c od*) as a rudimentary dorsal pouch (*d pch*).

PLATE 3.

Figs. 13-22.—*Locustana*, fifth (last nymphal) instar. Cross-sections from a series proceeding cephalad. $\times 40$.

Fig. 13.—Through base of lateral ovipositor lobes showing tips of inner

ovipositor lobes (*i ovip*), posteriorly reaching anterior ovipositor lobes and tip of egg-guide (*eg*).

Fig. 14.—More anteriorly showing inner ovipositor lobes fusing with lateral ovipositor lobes and enclosing a groove (*gr 1*).

Fig. 15.—More anteriorly showing accessory genital invagination from the ninth sternum (*acc inv 1*), anterior ovipositor lobes, subgenital plate (eighth sternum) with dorsal median egg-guide (*eg*).

Fig. 16.—More anteriorly showing spermathecal invagination (*spm inv*) between anterior ovipositor lobes.

Fig. 17.—More anteriorly through segment 8 showing lateral pouches from floor of genital cavity (*l pch*).

Fig. 18.—More anteriorly through same showing common oviduct opening by means of gonopore (*go*) on floor of genital cavity.

Fig. 19.—A few sections more anteriorly.

Fig. 20.—More anteriorly showing genital cavity prolonged as a dorsal pouch (*d pch*) above common oviduct.

Fig. 21.—At junction of seventh and eighth sterna showing paired mesodermal oviducts (*mes od*) opening into common oviduct (*c od*).

Fig. 22.—Through posterior region of seventh segment showing mesodermal oviducts passing dorsally.

Figs. 23-5.—*Blattella*, newly hatched first instar nymph. Cross-sections proceeding cephalad. $\times 150$.

Fig. 23.—Through ninth sternum showing rudiments of lateral ovipositor lobes (*r-l ovip*) and bases of styli (*b-st*) lateral to these.

Fig. 24.—Through eighth sternum showing rudiment of anterior ovipositor lobes (*r-a ovip*).

Fig. 25.—Through posterior part of seventh segment showing ampullae (*amp*) of mesodermal oviducts (*mes od*).

Figs. 26-32.—*Blattella*, 2nd instar (late). Cross-sections from a series proceeding cephalad.

Fig. 26.—Through lateral ovipositor lobes. $\times 120$.

Figs. 27, 28, and 29.—Showing groove (*gr 1*) between lateral ovipositor lobes becoming a tubular invagination anteriorly (*acc inv 1*) which soon gives off two diverticulæ (fig. 29). (The encroaching seventh sternum is cut before the eighth in fig. 29.) $\times 350$.

Fig. 30.—Through anterior ovipositor lobes. $\times 120$.

Fig. 31.—Through bases of anterior ovipositor lobes showing groove (*gr 2*) between them. $\times 120$.

Fig. 32.—Near junction of segments 7 and 8 showing common oviduct (*c od inv*) originating from floor of genital cavity (seventh intersternal membrane) and ampullae (*amp*) of mesodermal oviducts (*mes od*) lying on either side of it.

Fig. 33.—Forficula, third instar nymph. Median longitudinal section

showing spermathecal (*spm inv*) and common oviduct invaginations (*c od inv*). $\times 80$.

Figs. 34-6.—Forficula, second instar nymph. Cross-sections from a series proceeding cephalad. $\times 120$.

Fig. 34.—Through junction of eighth and ninth segments showing spermathecal invagination (*spm inv*) from eighth intersternal membrane.

Fig. 35.—At junction of seventh and eighth segments showing common oviduct invagination (*c od inv*).

Fig. 36.—A few sections more anteriorly, through posterior region of seventh segment showing common oviduct invagination ending blindly between ampullae (*amp*) of mesodermal oviducts.

PLATE 4.

Figs. 37-44.—Blattella, fourth instar nymph (early). Cross-sections from a series proceeding cephalad. $\times 80$.

Fig. 37.—Showing lateral and inner ovipositor lobes (*l* and *i ovip*) and encroaching seventh sternum with formation of large genital cavity (*gen cav*).

Fig. 38.—Through bases of lateral and inner ovipositor lobes showing groove (*gr l*) between latter.

Fig. 39.—More anteriorly showing accessory genital invagination (colateral gland, *acc inv l*). Note lateral folds arising from floor of genital cavity.

Fig. 40.—More anteriorly showing lateral attachment of bases of anterior ovipositor lobes (*a ovip*).

Fig. 41.—More anteriorly showing vestigial accessory genital invagination (*acc inv 2*) from groove between bases of anterior ovipositor lobes. Note common oviduct invagination (*c od inv*) opening on floor of genital cavity.

Fig. 42.—Near junction of seventh and eighth sterna showing ampullar ends (*amp*) of mesodermal oviducts lying ventrally on either side of common oviduct invagination.

Fig. 43.—A few sections more anteriorly showing paired origin of first pair of spermathecae (*spm inv 1*) from floor of genital cavity. Mesodermal oviducts proceeding dorsally.

Fig. 44.—A few sections more anteriorly showing paired origin of second pair of spermathecae (*spm inv 2*) from floor of genital cavity.

Fig. 45.—Colemania, 22 mm. (fourth instar of Locustana). Median longitudinal section showing relations of ovipositor lobes, common oviduct, genital invaginations. $\times 45$.

Fig. 46.—Blattella, fourth instar (late).—Portion of longitudinal section through genital segments, near midline, showing the subgenital plate enclosing a genital cavity (*gen cav*) and the relations of the genital invaginations to this. $\times 75$.

Figs. 47-9.—*Blattella*, sixth (last nymphal) instar. Cross-sections from a series proceeding cephalad. $\times 45$.

Fig. 47.—Through genital cavity showing folds with tracts of glandular cells (*gl c*) at their bases, and also ovipositor lobes.

Fig. 48.—Near anterior end of genital cavity showing common oviduct traversing fold formed by the invagination or pocket (*pct*) beneath it. Note first pair of spermathecae (*spm 1*) and the spermathecal ducts (*spm dct 1* and *2*).

Fig. 49.—More anteriorly showing mesodermal oviducts (*mes od*) opening into common oviduct invagination. Laterally lie the second pair of spermathecae (*spm 2*).

Fig. 50.—*Blattella*, sixth instar (early).—Portion of longitudinal section through genital segments, near midline (slightly oblique), showing spermathecal and common oviduct invaginations from floor of subgenital plate (seventh intersternal membrane), &c. $\times 65$.

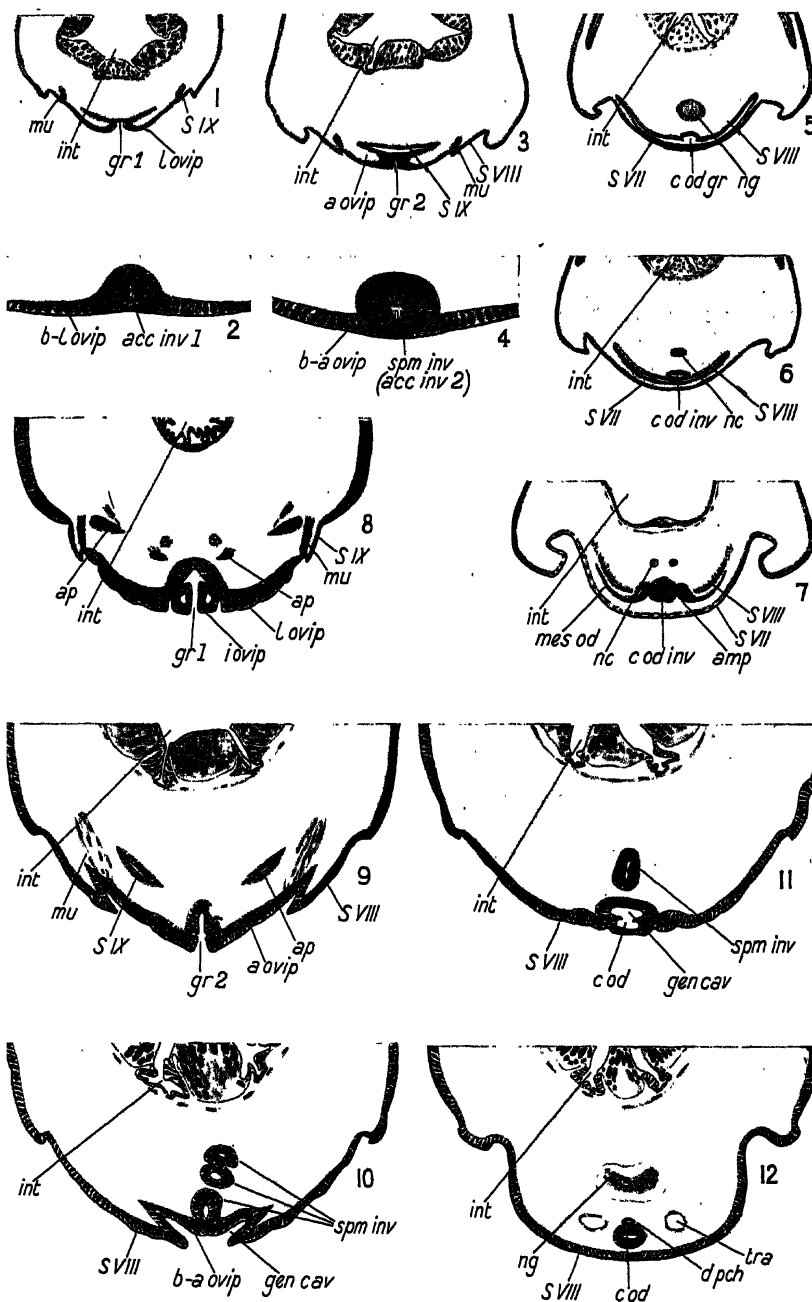
Figs. 51-4.—*Blattella*. Adult female (newly moulted). Cross-sections from a series proceeding cephalad. $\times 45$.

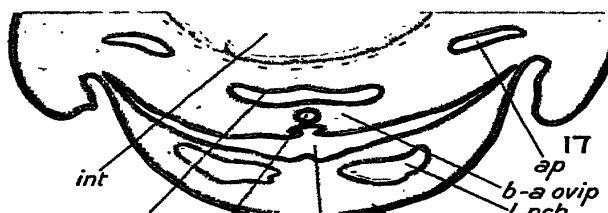
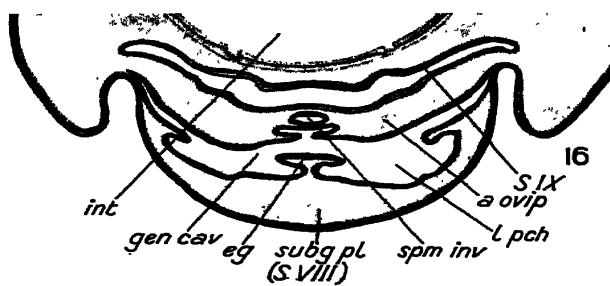
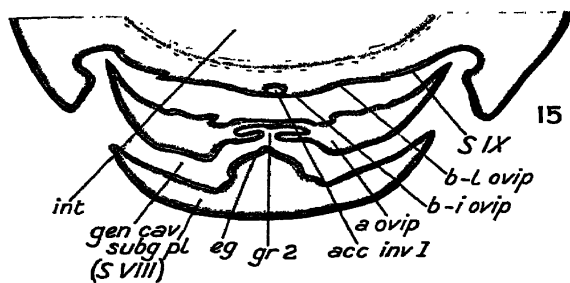
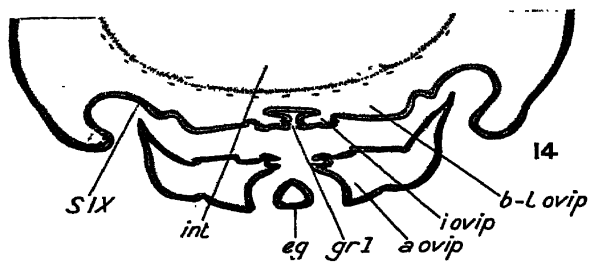
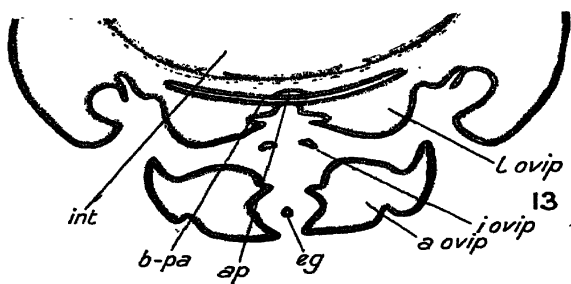
Fig. 51.—Through genital cavity showing lateral folds (*l fld*), also ovipositor lobes.

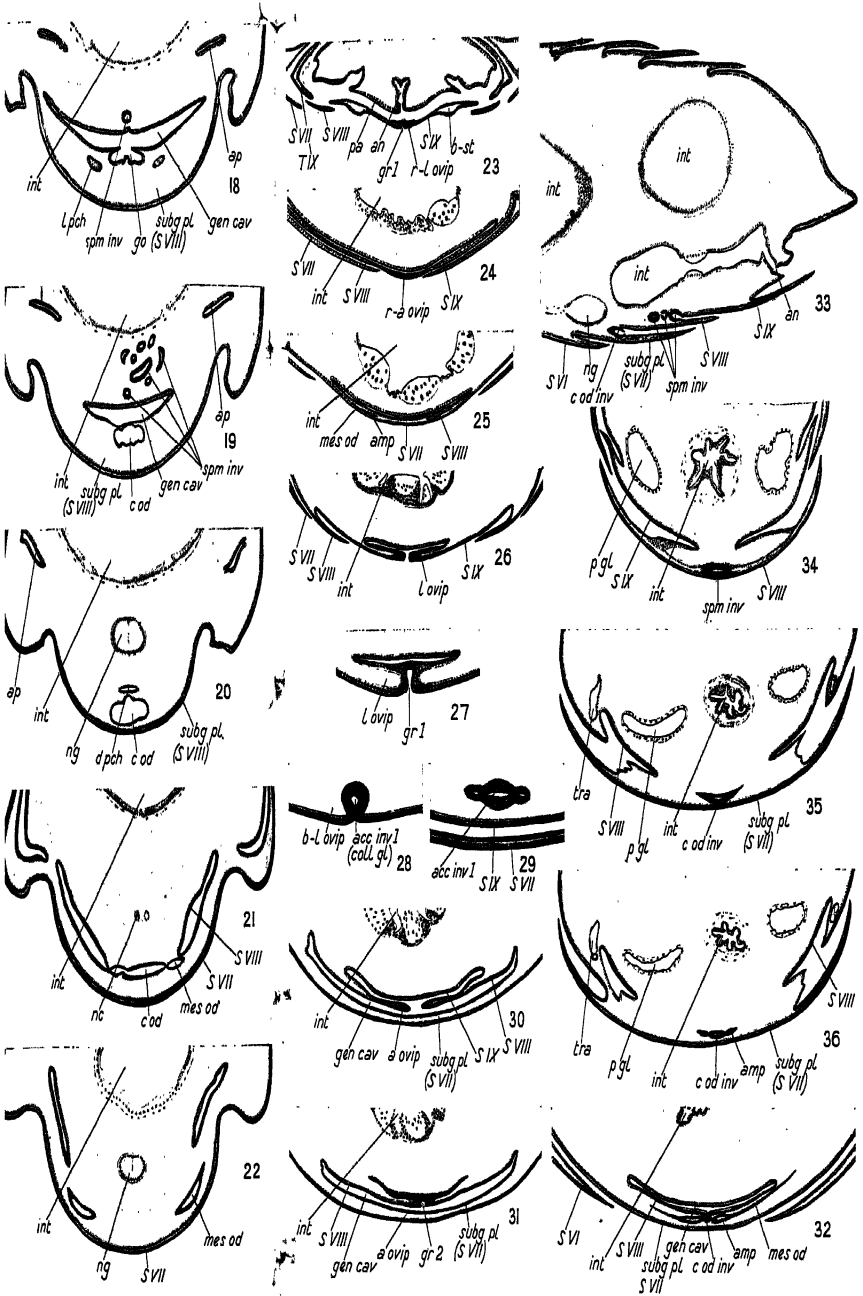
Fig. 52.—More anteriorly through genital cavity and ninth segment showing common opening (*o coll gl*) of colleterial glands (*coll gl*) between bases of inner ovipositor lobes.

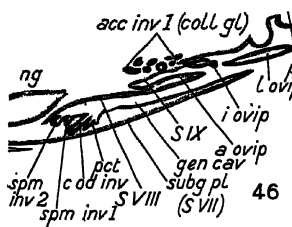
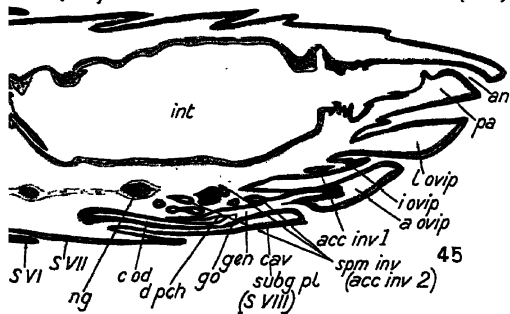
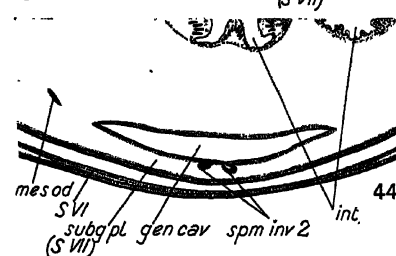
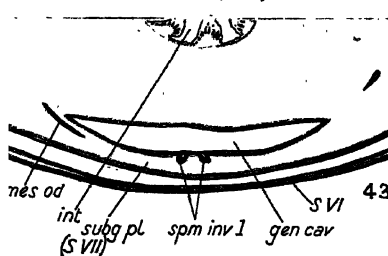
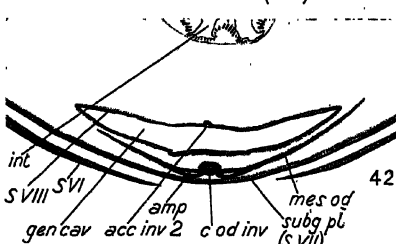
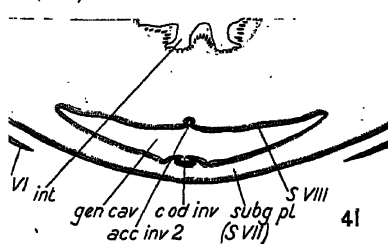
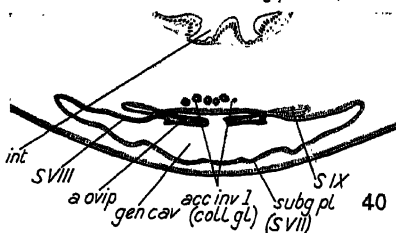
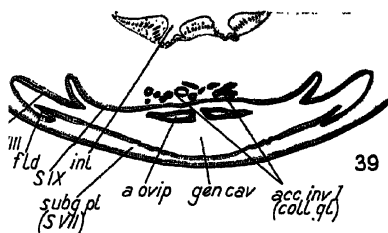
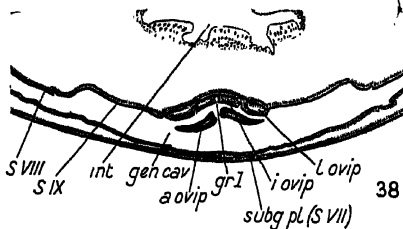
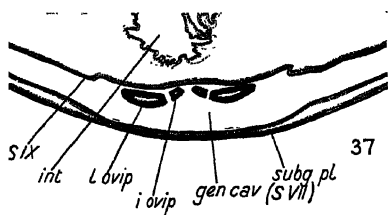
Fig. 53.—More anteriorly showing median longitudinal tract of glandular cells (*sgl*) marking position of atrophied accessory genital invagination between bases of anterior ovipositor lobes. Note dorsal sperm-guiding groove (*spm gr*) on fold carrying common oviduct.

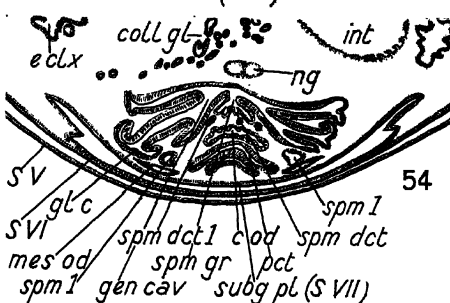
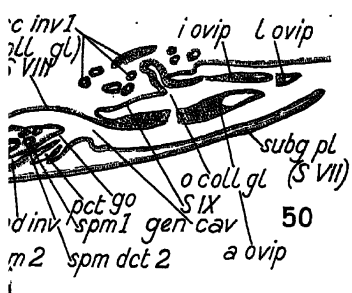
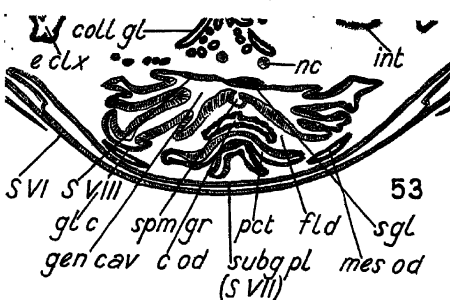
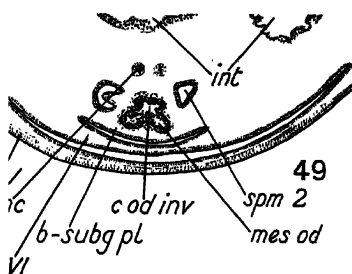
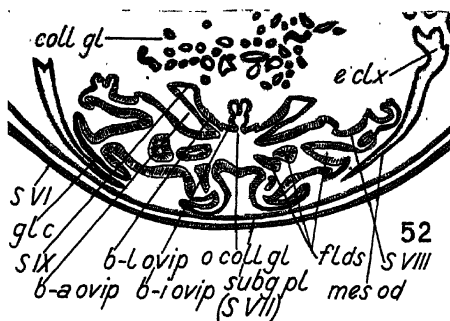
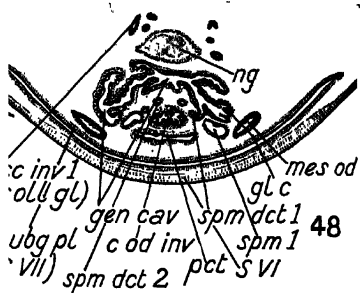
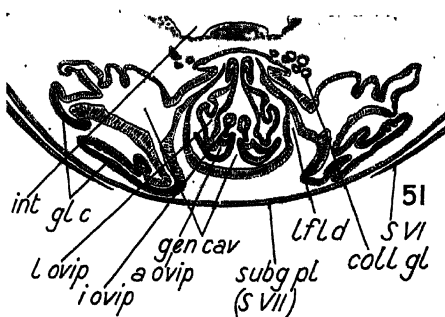
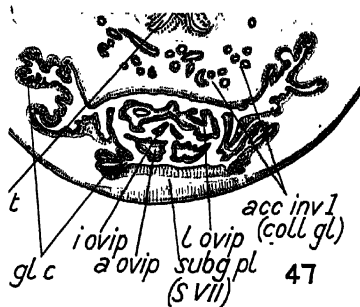
Fig. 54.—A few sections more anteriorly showing openings of spermathecal ducts (*spm dct 1* and *2*) into sperm-guiding groove (*spm gr*); also common oviduct, first pair of spermatheca (*spm 1*) and paired oviducts (*mes od*).











On the Development of the Mid-Gut in the Larval Stages of *Vanessa urticae* (Lepidoptera).

By

H. Henson, B.Sc.,

Assistant Lecturer in Zoology in the University of Leeds.

With Plate 5 and 1 Text-figure.

CONTENTS.

	PAGE
1. INTRODUCTION	87
2. TECHNIQUE	88
3. STRUCTURE AND DEVELOPMENT OF THE MID-GUT	89
(a) First Instar	89
(b) Second Instar	92
(c) Third Instar	93
(d) Fourth Instar	95
(e) Fifth Instar	97
4. CORRELATION WITH PREVIOUS WORK	100
5. SUMMARY	102
6. BIBLIOGRAPHY	103
EXPLANATION OF PLATE	104

1. INTRODUCTION.

WHILST examining sections of Lepidopterous larvae, with a view to following some of the main features of their metamorphosis, it was forcibly impressed upon me that an accurate interpretation of the structure of the mid-gut was impossible. Examination of available literature merely served to emphasize the confusion of ideas. The present work was accordingly undertaken, large numbers of larvae being collected, and the problem studied by the ordinary methods of micro-technique.

Acknowledgements.—My warmest thanks are due to Professor H. M. Fox of the University of Birmingham for the

facilities granted to me in the prosecution of this work. I must further acknowledge my indebtedness to Mr. L. Eastham for guidance and encouragement during the early stages of the work. Lastly, my best thanks are due to Dr. A. D. Imms, who allowed me to finish this work at the Entomological Laboratories of the Rothamsted Experimental Station.

2. TECHNIQUE.

The technique developed was such as to give good histological detail. A cytological study was not attempted. The earlier results were often far from satisfactory in spite of great variation in fixing methods. Fixing fluids tried included Flemming's, chrome-osmic, picro-chlor-acetic, Carnoy's, acetic-alcohol, and 10 per cent. formalin. The double-embedding method using celloidin and wax was later adopted and thereafter the preparations were greatly improved and were sometimes extremely good. I would here acknowledge my indebtedness to Mr. H. G. Newth for much technical assistance and advice. The fixatives giving best results were Carnoy's fluid and 10 per cent. formalin. This latter gave surprisingly good fixation as a rule, provided it was followed immediately by 70 per cent. or 90 per cent. alcohol. Whole larvae (not dissected-out intestines) were employed, since it is hoped to investigate other organs in the same material in a subsequent paper. The staining methods were varied to include Delafield's haematoxylin, Heidenhain's iron haematoxylin, Mayer's acid haemalum, and haematein (in 70 per cent. alcohol), but eventually only the iron haematoxylin and the acid haemalum were persevered with. Counterstains employed include eosin, orange G, and aurantia. Eosin gave its best results when used in aqueous solution and then differentiated in 70 per cent. alcohol containing a trace of orange G. It was found advisable to keep the counterstaining very feeble.

The material was collected on several field expeditions. Several large batches of eggs were also found. The instars were found to be five in number and the larvae had a characteristic head-width in each instar. The maximum deviation from the

average in each instar never rose to a value which made confusion possible. The characteristic widths were found to be 0.38 mm., 0.69 mm., 1.18 mm., 1.95 mm., and 2.67 mm. respectively in the five instars.

In most cases whole or half larvae were cut transversely and longitudinally. The longitudinal series of course had some sections in which the gut was cut tangentially. These were of the greatest value, especially in the taking of cell measurements. In ordinary transverse sections of the gut it was not always possible to be absolutely sure of the cell limits, especially as it proved impracticable as a rule to cut at less than 8μ . It was here that the tangential sections proved their value, as it was possible to follow individual cells through several sections. The information thus obtained rendered the interpretation of the transverse sections certain.

3. STRUCTURE AND DEVELOPMENT OF THE MID-GUT.

(a) The First Instar.

In a newly hatched larva the mid-gut is relatively very large, being 138μ in diameter; the whole larva is 193μ in the same diameter (dorso-ventral). The gut lumen contains only a little plasma-like material, probably the remains of the egg-yolk. The epithelium consists of cells about 23μ high, carrying a striated border 1.5μ high. There are three kinds of cells present (fig. 1, Pl. 5), ordinary columnar epithelial cells, goblet cells, and very scattered, infrequent interstitial cells. The columnar cells are the ones possessing a striated hem. They are not particularly specialized and are $8-10\mu$ in diameter. The nucleus has the form of a chromatic mass immersed in a large vacuole. Hertig (1928) describes a similar state in living gut cells of adult honey-bees. The goblet cells have the same form as the columnar cells, but the upper half of each is occupied by a typical goblet. The nucleus is contained in the basal mass of cytoplasm. In one individual the goblets are $3-5\mu$ in diameter, but in another they may be 7μ . This is taken as evidence that they are in process of formation and that differentiation is pro-

ceeding. Later in the instar the increase in size is very marked. The contents of the goblet have the same affinity for counter-stain as the striated border and appear to consist of a closely packed mass of fibrils, in fact they are not optically distinguishable from striated border. Neither in this nor later stages has it been possible to determine the exact fate of the goblet cells or of their contents. As new goblet cells are undoubtedly added periodically, and their total number does not appear materially to increase, there must be some mode of disappearance, but it has not been possible to trace this as yet.¹ On the whole the goblet cells tend to be slightly smaller than the columnar cells. The interstitial cells are not very numerous; their bulk is mainly nucleus; this latter is usually $2-3\mu$ in diameter. It seems highly probable that the interstitial cells are really embryonic rudiments (i.e. sister cells of the ordinary epithelial cells). This is the mode of origin ascribed to apparently similar cells in both *Calliphora* and *Polistes* by Pérez (1910 and 1912). Only a further study of the late embryonic stages can definitely prove this.

During the first instar very considerable changes in the structure of the gut occur (fig. 2, Pl. 5). First the gut becomes about 250μ in diameter, whilst its constituent cells have nearly doubled in size. The fact that the total diameter of the gut increases in the same ratio as the size of the cells proves that few, if any, new cells have been added.

The columnar cells are usually cylindrical and in tangential sections are shown to be about 15μ in diameter. In a few cases, however, they are clearly elongated along the antero-posterior axis of the gut and have measurements of the order of $22\mu \times 12\mu$. The nuclei are ovoidal and roughly about 10μ across.

The goblet cells are also about twice as large as at the beginning of the instar. They have the same general features, but

¹ Fig. 4, Pl. 5, represents an unusual appearance which seemed at first to indicate that a goblet cell might be converted into a columnar cell by the eversion of the goblet, or that a columnar cell might be converted into a goblet cell by invagination of the striated border. Further study convinced me that neither of these processes occurred (v. pages 101-3).

the goblet extends much farther into the cell; the basal cytoplasm occupies $10-20\mu$ in a cell about 40μ high. The goblets appear to have a definite opening into the lumen and to contain a closely packed mass of fibrils, again indistinguishable from the striated border fibrils. Both these latter and the contents of the goblets are about twice as long as at the beginning of the instar. The goblets as seen in tangential sections are about 14μ across; the nucleus of these cells is about 5μ in diameter, so that it has not increased in size to anything like the same extent as the cell as a whole.

The interstitial cells are very numerous and form a complete network, in the meshes of which stand the columnar and goblet cells. The nucleus is well marked and is about 5μ across. Cell limits are not easily discerned, and—probably owing to recent nuclear division—are not yet present in many instances (vide fig. 2, Pl. 5). It is noteworthy that whilst having very largely increased in numbers they have also increased in size in about the same ratio as the rest of the gut.

Examining the data obtained in the light of the later stages described below, it would appear that the development of the mid-gut during the first instar is merely the continuation of embryonic processes. A stable condition is reached only at the very end of the instar. After this latter point has been reached the changes which occur are cyclical, and repeated in each instar, except the last. The evidence on which this view is based is (1) the general increase in the size of the cells to a point which is then more or less constant throughout larval life (vide table, p. 98), and (2) the absence of any fully differentiated cells during the early part of this first instar. Thus the true point of division between embryonic and larval stages is the end of the first instar, and not the moment of hatching as would be imagined *a priori*. The first effect of the intake of plant food is apparently to produce a great increase in the size of the cells. This may be due to water absorption from the leaf material eaten.

(b) The Second Instar.

The earliest phase yet seen in this instar shows quite plainly that round about the period of the ecdysis much cell differentiation has occurred. In main essentials, however, the gut has the same features as at the end of the first instar: there are interstitial cells, columnar cells of very varied sizes, and goblet cells of two definite different sizes.

The mid-gut is approximately 300μ in diameter anteriorly and 350μ posteriorly. There is thus an increase in diameter of roughly one-fifth to two-fifths, i.e. from 250μ to 300μ or 350μ , over the diameter noted at the end of the first instar. Whilst a few of the columnar cells are definitely larger than at the end of the first instar, it seems more likely that the increase in size is due to growth and differentiation of interstitial cells. In this latter case the cells can be spoken of as being added to the gut from the interstitial nests.

The interstitial cells are very definite (fig. 3, Pl. 5) and have nuclei which vary in size between 5μ and 3μ . Mitotic figures are to be observed.

From these interstitial cells there are many gradations in size up to the largest columnar cells. Many of these may be young goblet cells, but no feature has been found which will distinguish a young goblet cell from a young columnar cell. Most of the columnar cells are $10-17\mu$ in diameter, i.e. they are unaltered from the previous instar. There are obviously two different sizes of goblet cells present. One of these has the goblet $10-17\mu$ in diameter (about the same as in the late first instar), and the other has the goblet about $5-7\mu$ across. It is presumed that these latter are newly differentiated from interstitial cells. The contents are unchanged and still appear to be closely similar to the striated border. At a later stage in this instar development can be seen to have proceeded much further (fig. 5, Pl. 5). The interstitial cells are very numerous and show many mitotic figures. The nucleus is only $2-3\mu$ in diameter. We saw that earlier in the instar these nuclei were $3-5\mu$ in diameter, and at the end of the first instar were all about

5 μ across. It is thus to be concluded that reduction in size accompanies the increase in numbers in this instar. The columnar cells are not very different from the two previously described phases, although there may be more of the very large ones. A few typical measurements as taken in cross-sections of the cells are 20×34 , 28×24 , 13×13 , 8×12 , 18×13 , 17×13 (all in μ). Many of these cells, especially the larger ones, have vesicles attached to their lumen ends. These seem very similar in appearance to what are usually described as secretion globules (Bordas, 1911; Deegener, 1909). In this case I prefer to regard them as due to disintegration of the cells. The reasons for adopting this view may be stated as follows: (a) Feeding is continuous throughout both first and second instars. If these vesicles were digestive secretion, their production should also be either continuous or in rapidly alternating cycles. They have not been observed in any stage earlier than the late second instar. (b) The production of these vesicles has only been definitely observed from the very large columnar cells and not from the small ones. (c) The vesicles may contain portions of the nucleus or all of it.

Van Gehuchten (1890), who seems to be the main authority quoted in support of this view of gut secretion, also makes the latter observation. As the main concern of this paper is not to investigate secretion, the question will be discussed further in the concluding part.

The goblet cells are practically unchanged from the condition described for the earlier part of the instar. They still show two definite size categories, large with goblets 10–17 μ across, and small with goblets only 7 μ across.

(c) The Third Instar.

At a stage shortly after the ecdysis between the second and third instar it can easily be seen that cells of all sizes are present. The small and intermediate sizes are arranged in groups and have obviously been derived from nests of interstitial cells (fig. 6, Pl. 5). Many of the medium-size cells have small goblets within them and are interpreted as newly formed goblet cells.

A large amount of granular matter is to be seen between the cells and the peritrophic membrane. This is derived from the break-up of the above-mentioned vesicles. As in the last case, where these vesicles can be seen attached to cells, it is the very large columnar cells which are concerned. As before, this is taken as evidence of cell disintegration. The largest columnar cells present may be $20-30\mu$ in diameter and have correspondingly large nuclei. The goblet cells again show two definite size categories. In one of these the goblet is 10μ in diameter and in the other it is $5-6\mu$ in diameter (nuclei 7μ and 4μ respectively). In a few instances the goblet extends to the very bottom of its cell. These facts are interpreted as follows: the smallest goblet cells have been newly formed from interstitial cells in the period just following the ecdysis. The larger ones, 10μ across, are the same as the cells described at the end of the second instar as being $5-7\mu$ across; they have further differentiated during this period. The very large goblet cells found at the end of the second instar are possibly represented by the very few large ones in which the goblet extends to the very bottom of the cell. Most of their sister cells have disappeared in some manner undetermined (vide table, p. 98).

At a slightly later stage in the instar the various kinds of cells become very clearly marked off from one another (fig. 7, Pl. 5).

The interstitial cells are now very numerous, have nuclei about 5μ across, and show a few mitotic figures. The distinction between these cells and all others is such as to prove that no differentiation of these cells is now taking place. This is taken to indicate that cell differentiation only occurs just after the ecdysis and the period immediately following. During the remaining part of the instar cell division constitutes the chief if not sole activity of these cells.

The columnar cells vary considerably in size and are often drawn out in the direction of the long axis of the gut. Typical measurements are 12×12 , 60×12 , 36×15 , 14×8 , 30×14 , 55×8 (in μ). In cases like this it is essential that all cell measurements should be taken on tangential sections to avoid variation due to direction of section. Some of these big cells appear to

have a fragile nucleus which may be as much as 30μ in diameter. The goblet cells do not obviously fall into different size categories, and are in most cases $8-12\mu$ across the goblet. At the anterior end of the gut most of the goblets pass to the bottom of the cell, but at the posterior end they only reach half-way down. This is taken as meaning that the young goblet cells formed at the beginning of the instar have developed at a rapid rate and have caught up with more developed ones present from the end of the second instar. The contents of the goblets are still indistinguishable from the striated border.

In the resting period immediately preceding the next ecdysis the gut shrinks considerably in diameter, the cells become laterally compressed, tall and narrow. So much is this the case that most of the very large columnar cells obviously bulge into the lumen. The goblet cells are long and narrow, the goblets being $8-10\mu$ in diameter by 35μ in length. They do not in many cases extend to the bottom of the cell. The interstitial cells are very numerous and extend far upwards between the bases of the epithelial cells. In most cases their nucleus is about 5μ in diameter. There are no vesicles or granular matter in the gut at this stage.

Examination of these three stages shows quite definitely that during the third instar the following phenomena occur: large numbers of interstitial cells differentiate at the beginning of the instar, some cell disintegration occurring at the same time. Further development of these cells proceeds throughout the instar until they catch up with older cells carried over from the previous instar. The interstitial cells continuously divide until very great numbers are present at the end of the instar.

(d) The Fourth Instar.

Three different stages in this instar have been examined. The first of these is earlier in the instar than any previously described phases and shows quite definitely that cell differentiation commences only after the casting of the skin. The second stage is comparable to the stages described first in previous

instars. The third is exactly comparable to the later stages of the other instars.

The first is taken immediately after ecdysis (fig. 9, Pl. 5). An extremely large number of cells are present. In tangential sections columnar cells of various sizes and goblet cells almost uniformly 10μ across can be seen. These are separated from each other by large numbers of interstitial cells. There is a complete gradation in size from the smallest of these to the largest columnar cells. It is therefore presumed that cell differentiation is rapidly proceeding. No small goblet cells have yet been formed, but as in the stages described at the end of the third instar, many at the hind end of the gut only extend half-way or less down the cell, although $8-10\mu$ in diameter. Some of the interstitial cells are already beginning a new cycle of divisions since one mitotic figure has been observed.

The second stage examined in this instar is fairly similar to the earliest stages seen in the previous instars. Differentiation has further proceeded. Fig. 10, Pl. 5, shows definitely two different sizes of goblet cells. One of these is roughly $5-6\mu$ in diameter and the other $12-18\mu$. The smaller ones must be newly differentiated and the larger ones must have been carried over from the previous instar and correspond to those described as being $8-10\mu$ across at that time (cf. table, p. 98). The columnar cells are of many sizes, the largest being of the order of 55μ along one diameter; many are cylindrical and about 20μ across. There are many smaller ones arranged in groups round the larger ones, thus suggesting their recent formation from interstitial cells (cf. also the arrangement of the young goblet cells in fig. 10, Pl. 5). The interstitial cells are not very numerous, have nuclei $2-3\mu$ across, and show a few mitotic figures.

The latest stage examined in this instar shows a condition similar to that illustrated for the third instar in fig. 7, Pl. 5. The interstitial cells are numerous, well marked, and show occasional mitotic figures. The columnar and goblet cells are well defined and differentiation of new cells has ceased. The interstitial cells have nuclei almost uniformly 4μ across. As

before, the columnar cells may be 10μ in diameter and cylindrical, or plate-like and 50μ along their longest diameter. As in the previous phase, the goblet cells are sharply divisible into two size categories, one $5-8\mu$ in diameter and the other $11-18\mu$. This is taken as meaning that the small ones have enlarged slightly whilst the larger ones have not altered. The goblets are still lined by material which is optically indistinguishable from striated border.

Before describing the changes undergone by the gut during the fifth instar, it would perhaps be advisable to recapitulate the main results achieved up to this point. The fifth instar is different and complicated by the approach of the metamorphosis.

We have seen that the first instar is characterized by a continuation of embryonic development. The gut reaches its full differentiation only at the end of this instar. At each ecdysis subsequent to hatching, i.e. first to third, many new gut cells are added from interstitial cells, which then spend the rest of the instar recuperating their numbers. Put in another way, each of the second, third, and fourth instars has a cycle of development which may be divided into two parts: (1) an early one in which cell differentiation rapidly proceeds, and (2) a continuous period throughout the instar in which interstitial cells are dividing; this process is very rapid at the end of the instar when differentiation has ceased. These two periods may have some connexion with the two growth-rates per instar described by Yagi (1926).

A table summarizing the cell measurements and indicating the cell sizes will be found on p. 98.

(e) The Fifth Instar.

The main features of the gut as observed during this instar are really preliminary to metamorphosis. In nature they are, I believe, essentially processes of cell disintegration. It has to be borne in mind that there are two factors to be taken into account: disintegration processes which are actually part of metamorphic changes, and disintegration processes like those

Cell.	First Instar.		Second Instar.		Third Instar.			Fourth Instar.		
	Early.	Late.	Early.	Late.	Early.	Late.	Very Late.	Very Early.	Early.	Late.
Interstitial cells. Size of nucleus (diameter).	Few 2-3 μ	Many 5 μ	Fairly numerous 3-5 μ	Very numerous 2-3 μ	Few —	Numerous 5 μ	Very numerous 5 μ	Few 5 μ	Few 2-3 μ	Fairly numerous 4 μ
Goblet cells.	One size	One size	Two sizes	Two sizes	Three sizes (very largest rare).	One size	One size	One size	Two sizes	Two sizes
Diameter of goblet.	7 μ ———→	14 μ ———→	10-17 μ → 5-7 μ →	10-17 μ → 7 μ →	Very large 10 μ → 5-6 μ	8-12 μ —→	8-10 μ —→	10 μ ———→	12-18 μ → 5-6 μ →	11-18 μ 5-8 μ
Columnar cells. Diameter of cell.	Uniform size. 8-10 μ	Uniform size. 15 μ	Somewhat variable. 10-17 μ but some larger	Very variable. Up to 30 μ	Very variable. Up to 30 μ	Very variable. Up to 60 μ	Very variable.	Very variable.	Very variable. Up to 55 μ	Very variable. Up to 50 μ
Granular matter in gut.	Absent	Absent	Absent	Present	Present	Absent	Absent	A little present	Present	Present

Arrows indicate what are thought to be sequences in development.

described in previous instars. Owing to the fact that suitable stages of *Vanessa* were not obtained, the last two stages were taken from material of *Pieris brassicae*. This does not appear to affect the issue materially.

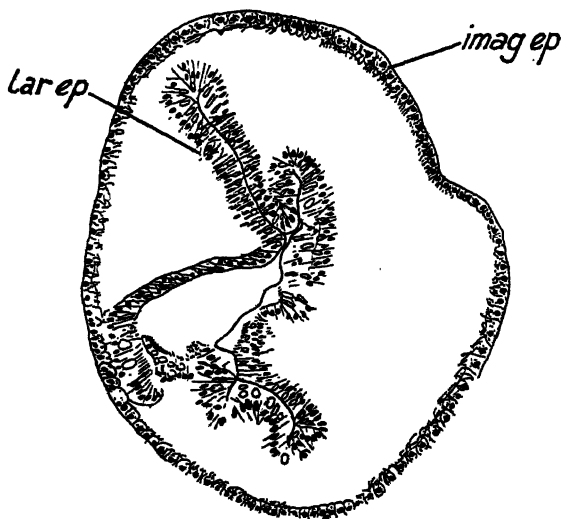
Quite early in the fifth instar notable changes can be seen to have occurred (figs. 11 and 12, Pl. 5). The interstitial cells are now of rounded form and very considerably larger than in previous instars. They have the form and appearance of the imaginal gut cells, and will be called by this name from now onwards. These cells may be $20\mu \times 12\mu$, their nuclei 10μ , in diameter. They are not nearly so numerous as at subsequent stages, but their division has not been observed. It is believed that they arise from interstitial cells, some of which remain behind (fig. 13, Pl. 5). The contents of the goblets appear to have shrunk somewhat away from the cell walls (fig. 12, Pl. 5). They consist of a very darkly staining central mass (not always present) surrounded by the fibrillar material previously described. These are interpreted as changes incidental to metamorphosis. The fibrillar material is much less obvious than in previous phases. This may account for the failure of those other authors whose work was mainly confined to later stages to realize the significance of these appearances. The columnar cells appear to be much more uniform in size than in any previous instar, except the first. They are $12-15\mu$ in diameter and about 60μ high. The nuclei are pear-shaped and $10\mu \times 20\mu$ or thereabouts. Many of these cells have granular protrusions attached to their lumen ends, whilst many vesicles are to be seen free in the lumen (fig. 11, Pl. 5). These are also interpreted as changes indicating cell disintegration preparatory to metamorphosis.

A much later stage taken from *Pieris brassicae* material shows essentially the same features but much more pronounced. There is a large amount of granular matter in the gut, whilst the goblets of the goblet cells are represented only as a shapeless darkly staining mass (fig. 13, Pl. 5). The number of imaginal cells has greatly increased, so that they now form an almost complete epithelium underneath the larval gut cells.

The last stage is shown in Text-fig. 1. It merely represents

the casting off wholesale of the larval cells into the gut lumen where the processes of disintegration become complete. The imaginal gut cells are left behind to form a new epithelium. These changes, however, belong to the metamorphosis proper and have been dealt with by Deegener (1908) in *Malacosoma castrensis*.

TEXT-FIG. 1.



Imag. ep. = imaginal epithelium.
Lar. ep. = larval epithelium.

4. CORRELATION WITH PREVIOUS WORK.

It is not proposed here to deal with the whole of the vast literature on the insect mid-gut. Good bibliographies are to be found in most of the papers cited at the end of this work.

The earliest paper which need concern us is undoubtedly that of Van Gehuchten (1890). It is important because it is the foundation of the current idea that the granular protrusions seen on the epithelial cells are secretion vesicles. A critical examination of this paper shows that the thesis is far from being proved. Statements appearing in the paper are: 'the gut has

two definite kinds of cells, secreting and absorbing'; 'the secreting cells give rise to globular protrusions which are the secretion product'. Other statements which appear to me to be of considerable importance are the following: 'when these globules are present the cell has no striated border'; 'about the time of metamorphosis numerous small cells appear in rows round the bases of the epithelial cells'; 'in living animals the secretion is clear, not granular—it has not been possible to analyse the globules, but they are obviously rich in albuminoids'. There is no mention of the particular phase of the life-history dealt with, except that larvae in which the gut had cleared preparatory to metamorphosis were never used. Van Gehuchten further states that the nucleus is sometimes carried out with the secretion and that the cell then dies, to be replaced from small cells found low down on the basement membrane. It must be admitted that none of these statements is really inconsistent with the idea that the phenomena are due to cell disintegration; at least it cannot be pretended that the existence of an act of secretion has been proved. Balbiani (1890) in his work on the Myriapod *Cryptops* does not go beyond what is warranted by the observations. He also finds two kinds of cells: (1) ordinary epithelial cells and (2) mucous or caliciform cells (presumably=goblet cells). As in the present work, he finds that these latter arise from interstitial cells. They enlarge during development and become full of mucus, but they have never been seen to discharge.

Later authors have taken Van Gehuchten's work as standard and have made no attempt to prove that the process is really one of cell secretion. They have been mainly concerned with the question as to whether the goblet and columnar cells are dimorphous or merely different functional phases of the same cell. Vignon (1901) says that the goblet cell is not a phase in the development of the cylindrical cell. Deegener (1909) could not decide (in *Deilephila euphorbiae*) whether they were or were not functional phases of the same cell. He ascribed a secretory function to both kinds of cells and attempted to correlate the appearance of the cells with periods of eating and fast-

ing. He was using chiefly larvae of the last instar. Bordas (1911) again states that the 'vésicules saillantes' are secretion products. Hufnagel (1918) gives an account of some larval stages in *Hyponomeuta padella* with which the present paper agrees fairly well.

Pavlovsky and Zarin (1922), working on the honey-bee, say that the formation of bladders is artifact. Newcomer (1914) also thinks they may be artifact, but follows up the statement with: 'they are more likely to be drops of digestive fluid such as Van Gehuchten has described in *Ptychoptera*'. A few paragraphs on the silkworm, in the same work, agree well with my own findings.

Two of the most recent papers on this subject are by Shinoda (1926 and 1927). He also says 'that the secretion is emitted in the same manner as described by Van Gehuchten'. He then chooses arbitrarily a sequence in which a columnar cell emits secretion, becomes acidophile and develops a goblet; this latter stage is described as the second stage of senescence. This is at variance with my observations. Goblet cells are shown in this paper to be present at the time of hatching and to display from the first a striated border round the goblet cavity. Shinoda has overlooked these facts. He describes the goblet as a small quantity of acidophile cytoplasm with a goblet-like form. It is submitted here that the contents of the goblets cannot be described as cytoplasm. Shinoda further says that he did not find periodic recovery but continuous cell regeneration. It will be seen that the present paper postulates a periodic cycle in every instar. This agrees with Verson (1897), Tchang-Yung-Tai (1928), and Haseman (1910).

5. SUMMARY.

1. The mid-intestine of *Vanessa urticae* has three separate categories of cells, interstitial cells, goblet cells, and ordinary columnar epithelial cells.

2. The interstitial cells renovate the epithelium by the addition of new cells at each larval ecdysis.

3. There is a period at the beginning of each instar during which cell differentiation occurs (from interstitial nests). This

process soon ceases. Cell division by mitosis occurs amongst the interstitial cells throughout the instar.

4. No good evidence could be obtained that the goblet cells and columnar cells are homomorphous. The goblet cell cannot become a columnar cell because it undergoes a cycle of growth and differentiation which diverges increasingly from the columnar type. Similarly the goblet cells cannot be derived from senescent columnar cells because they are present at the time of hatching. Both are derived by independent modification of interstitial cells and are therefore dimorphous.

5. The goblet cells contain material which is optically indistinguishable from striated border.

6. It has been shown that in this animal the so-called merocrine method of secretion of the gut cells may not be a secretion process at all. The alternative view that the formation of 'secretion' vesicles is really a process of cell disintegration, due to wear and tear, or to the incidence of metamorphosis, is here favoured.

6. BIBLIOGRAPHY.

- Balbani (1890).—"Études anatomiques et histologiques sur le tube digestif des *Cryptops*", 'Arch. Zool. Exp.', 2^e sér., tom. vii.
- Bordas (1911).—"L'appareil digestif et les tubes de Malpighi des larves des Lépidoptères", 'Ann. Sci. Nat., Zool.', vol. i.
- Deegener, P. (1908).—"Entwicklung des Darmkanals der Insekten während der Metamorphose. Teil II, *Malacosoma castrensis*", 'Zool. Jahr. Jena', Bd. xxvi.
- (1909).—"Beiträge zur Kenntnis der Darmsecretion (*Deilephila euphorbiae*)", 'Arch. f. Naturgesch'.
- Frenzel (1886).—"Einiges über den Mitteldarm der Insekten sowie über Epithelregeneration", 'Arch. mik. Anat.', Bd. 26.
- Haseman (1910).—"Structure and Metamorphosis of the Alimentary Canal of the Larva of *Psychoda alternata*", 'Ann. Ent. Soc. Amer.', vol. iii.
- Hertig, M. (1923).—"The normal and pathological Histology of the Ventriculus of the Honey-bee, with special reference to Infection with *Nosema apis*", 'Journ. Parasit.', vol. 9, no. 3.
- Hufnagel (1918).—"La métamorphose de *Hyponomeuta padella*", 'Arch. Zool. Exp.', tom. 57.
- Newcomer (1914).—"Digestion and Digestive Epithelium in Insects", 'Ann. Ent. Soc. Amer.', vol. vii.

- Pavlovsky and Zarin (1922).—"On the Structure of the Alimentary Canal, and its Ferments in the Bee (*Apis mellifera*)", 'Quart. Journ. Micr. Sci.', vol. 66, N.S.
- Pérez, Ch. (1910).—"Recherches histologiques sur les métamorphoses des Muscides", 'Arch. Zool. Exp.', 5^e sér.
- Shinoda, O. (1926).—"Contributions to the Knowledge of Intestinal Secretion of Insects. I", 'Mem. Coll. Sci. Kyoto Imp. Univ.', Ser. B, vol. ii.
- (1927).—Ditto. II, 'Zeit. f. Zellforschung u. Mikr. Anat.', Bd. 5, 3. Heft.
- Tohang-Yung-Tai (1928).—"Les rénovations successives (partielles et totales) de l'épithélium de l'intestin moyen chez les chenilles de *Galleria mellonella*", 'Compt. Rend. Soc. Biol.', tom. xcviii.
- (1928).—Ditto, 'Bull. Soc. Zool. de France', tom. liii, Jan. 10 and Jan. 24.
- Van Gehuchten (1890).—"Recherches histologiques sur l'appareil digestif de la *Ptychoptera contaminata*", 'La Cellule', tom. 6.
- Verson (1897).—"L'évolution du tube intestinal chez le ver-à-soie", 'Arch. Ital. Biol.', tom. 28.
- Vignon (1901).—"Recherches sur les épithéliums", 'Arch. Zool. Exp.', tom. 9.
- Yagi, N. (1926).—"Analysis of the Growth Curves of the Insect Larvae", 'Mem. Coll. Agric. Kyoto Imp. Univ.', vol. i.

EXPLANATION OF PLATE 5.

LETTERING.

B.m., basement membrane; *Col.*, columnar epithelial cell; *Diff.*, differentiating cell; *Gob.*, goblet cell; *Gran.*, granular matter or vesicles in gut lumen; *Imag.*, imaginal cells; *Int.*, interstitial cells; *Mit.*, mitosis. All figures were drawn under the camera lucida Oc. Zeiss 8 C.P., obj. Zeiss 3 mm. apr.

- Fig. 1.—Transverse section of gut of newly hatched larva. Carnoy.
- Fig. 2.—Same at end of first instar. Carnoy.
- Fig. 3.—Same in early second instar. Carnoy.
- Fig. 4.—Small piece of gut of fourth instar larva. Carnoy.
- Fig. 5.—Two adjacent tangential sections of gut of late second instar larva. Carnoy. (Figure below is at a lower level, i.e. nearer to basement membrane, than the one above.)
- Fig. 6.—Transverse section, gut of early third instar larva. Carnoy.
- Fig. 7.—Same in late third instar.
- Fig. 8.—Transverse section of mid-gut of very late third instar larva (i.e. just before ecdysis). Carnoy.
- Fig. 9.—Same very early in fourth instar (i.e. just after ecdysis). Carnoy.

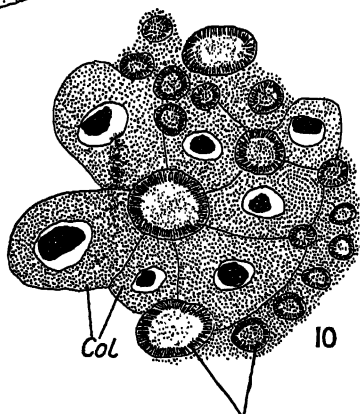
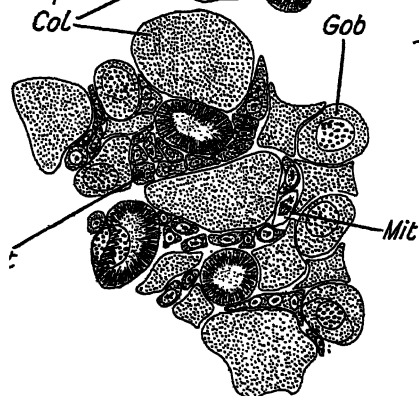
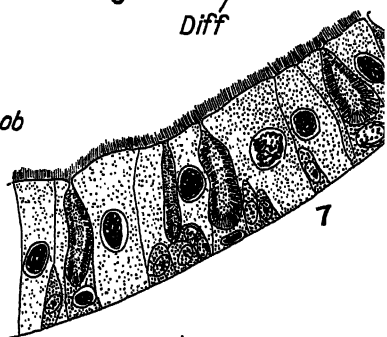
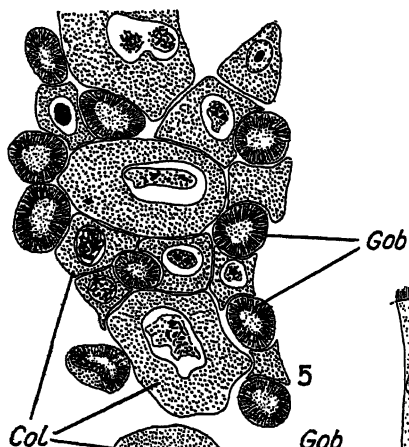
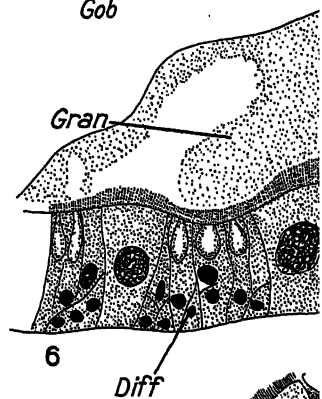
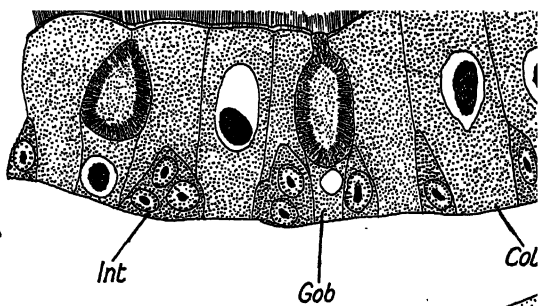
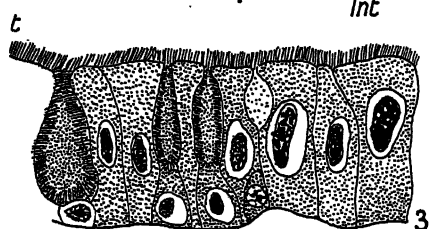
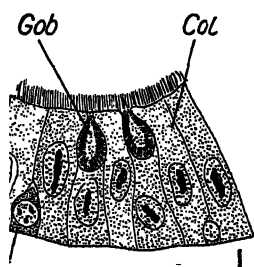
Fig. 10.—Tangential section of gut in early fourth instar. Carnoy. Note the arrangement of the small goblet cells in groups. A tendency towards the suppression of cell walls is to be noted in figs. 8 and 10. Compare this with a similar phenomenon described by Haseman (*Psychoda*) and Hufnagel (*Hyponomeuta*).

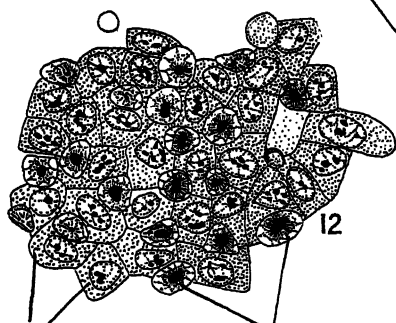
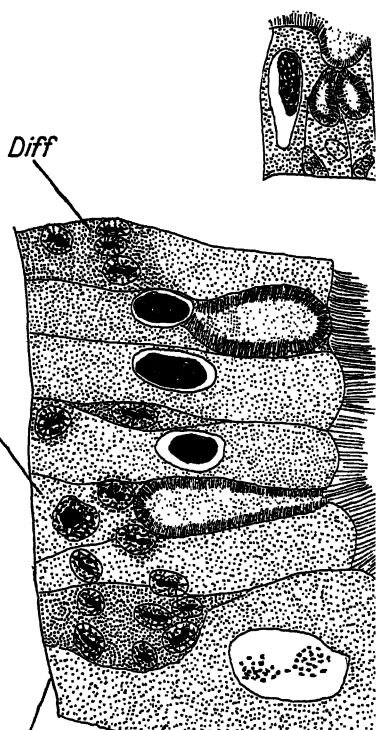
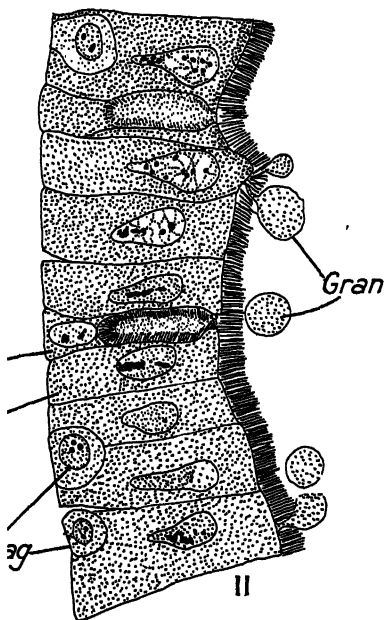
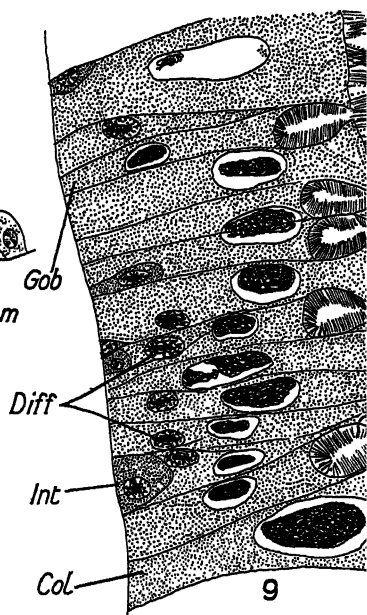
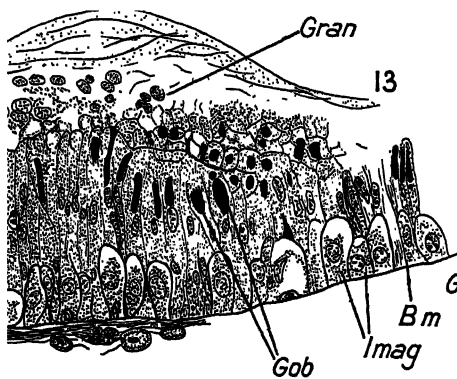
Fig. 11.—Transverse section of mid-gut in early fifth instar. 10 per cent. formalin.

Fig. 12.—Same in tangential section. 10 per cent. formalin.

Fig. 13.—Transverse section of late fifth instar in *Pieris brassicae*. Carnoy.

The apparent crossing of the fibres of the striated border in some of the figures is due to drawing in the depth of the section.





Physophaga sappheira, n.g., n.sp.

By

Muriel Percy.

With Plate 6.

IF *Gammarus pulex* can be caught in the act of moulting, the cast-off chitinous exoskeleton will often be found to contain a crowd of minute ciliates that dart to and fro with extraordinary rapidity. At this stage they are quite transparent and colourless, but in the course of a few minutes they begin to swell and turn faintly blue, and their movements become less violent. They remain for some hours in the moult, passing ceaselessly up and down inside the appendages and about the proctodaeum, increasing to three or four times their original size and often deepening in colour to a rich sapphire blue.

No work appears to have been done upon these ciliates hitherto, but their morphology and life-cycle evidently connect them with the genera *Polyspira* and *Gymnodinoides* described by Minkiewicz in 1912-13 from moults of marine decapods. Later investigations by Chatton have shown the characteristics of these genera to consist in the formation upon the host of resting cysts that open just before a moult, so that the ciliates on emerging can creep into the old exoskeleton and be thrown off with it.

The next phase, of distension and coloration, he proved to result from absorption inside the moult of a carotinoid albumen presenting features of special interest. Finding that *Polyspira* and *Gymnodinoides* showed structural resemblances to *Foettingeria* (Caullery and Mesnil, 1903), the ciliate of the coelenteric cavity in *Actinia*, Chatton proposed to include them, with other genera, in the family *Foettingeriidae*, and to consider this family as probably representing successive stages in the transformation of free-living ciliates into internal parasites or commensals.

It will be seen from what follows that the ciliate of *Gammarus* moults adds another genus to the Foettingeriidae.

MORPHOLOGY OF THE ADULT.

The mature specimens vary much in size, measuring 40–85 μ in length; they taper slightly in front, and are rounded posteriorly. Seen in ventral view, the outline is that of a broad bean, the right side nearly straight, and the left convex.

The ventral surface is rather flattened and has a small depression or dimple towards the right side (fig. 1, Pl. 6).

Bordering on this dimple is the small mass of protoplasm which contains the nuclei, the mouth, and the contractile vacuole.

The homogeneous substance absorbed from the crustacean moult fills the rest of the interior of the ciliate; it is sometimes quite pale in colour and sometimes an intense blue. The posterior part of this accumulation generally encloses a small collection of yellow granules and pigment which stain darkly with neutral red. Rare instances occur of moults containing ciliates in which the whole homogeneous substance is bright yellow instead of blue. It is traversed usually by three main strands of protoplasm which originate in the ventral protoplasmic mass. One of these passes to the dorsal surface, one to the anterior, and one to the posterior extremity. The latter is the most conspicuous, and encloses the posterior end of the macronucleus. These strands contain scattered granules that stain with neutral red; they spread into a thin surface film of protoplasm, forming broad bands beneath the rows of cilia; this surface layer contains granules which do not stain with neutral red, but show up clearly with Lugol's iodine solution. In well-fixed preparations they stain deeply with iron haematoxylin. There are eight bands of cilia besides the two rows that fringe the buccal groove. They are spirally twisted by the irregular form of the animal, and converge to a small apical extension of clear protoplasm which contains a few granules (see fig. 1, Pl. 6).

The Macronucleus is large, elongated, and elliptical in shape,

and generally has the chromatin disposed in large rounded granules of varying size, clearly visible in the living. But specimens often occur in which these large chromatin granules are not present, the nucleus being finely granular throughout. In the winter months, when the ciliates appear to lack vitality, this change in the nucleus is very noticeable.

The Micronucleus lies near the anterior end of the macronucleus and is very refringent. After fixation it stains intensely.

The Mouth (figs. 1 and 3, Pl. 6) lies in the angle between the micronucleus and the anterior end of the macronucleus, and is associated with a curious organ like that discovered by Caullery and Mesnil (1903) in *Foettingeria* and named by them the 'rosace'. Both the mouth and rosace are often extremely hard to see, and disintegrate quickly when the ciliate dies. The mouth is a small round opening through which projects a tuft of cilia.

The Rosace (fig. 3, Pl. 6) lies immediately below the mouth, apparently filling the gullet, and probably providing a means both of suction and filtration, as no solid particles seem ever to be ingested. Grains of indian ink can be seen collecting at the buccal orifice, but do not pass through.

When ammonium carminate solution is ingested it forms a coloured streak, extending from near the rosace to the posterior end of the central protoplasmic strand, where it accumulates.

In living specimens, seen from the ventral surface, the rosace appears as a refringent spot with refringent lines radiating from it. Sometimes vacuolar spaces appear between the lines and give the form of a flower with nine or more petals (fig. 3, Pl. 6). In one case each petal seemed to end in a refringent granule. The central spot sometimes seems to consist of granules in vibratory motion.

Seen through the body of the ciliate from the dorsal surface, the rosace is distinguishable as a small projection of the protoplasmic mass, ending in a knob from which lines radiate.

It can sometimes be fixed and stained, but good preparations are difficult to get. One film, fixed in Carleton's mercuric chloride and formalin mixture and stained with Ehrlich's

haematoxylin, shows the vacuolar 'petal' formation and the refringent spot, which is seen to lie towards the left centre of the rosace.

This organ will be referred to later in dealing with the young ciliates produced by schizogony.

The Buccal Groove. From the mouth the shallow buccal groove passes to the right and turns backward towards the posterior end of the ciliate, like that figured by Minkiewicz (1913) for *Polyspira*. The rows of cilia which line the edges of this groove extend round nearly half the ciliate's circumference, running parallel with the ciliary bands. The groove itself is difficult to distinguish, because all means hitherto found of slowing the ciliate's movements tend to cause distension of the cuticle and so obliterate the depression.

The Contractile Vacuole discharges into the buccal groove through a very small elliptical opening in the cuticle. This opening can often be plainly seen, and might be mistaken for the mouth, which is much more difficult to distinguish and lies some distance to the left of the contractile vacuole. Between these two openings there is a conspicuous tuft of cilia.

MULTIPLICATION CYSTS.

The ciliates leave the moult after a few hours and swim actively about in the water, always moving with the anterior end forward and revolving on the long axis.

They may encyst almost at once, or remain active for hours before doing so. When encystment is about to take place, they come to rest on the bottom of the capsule or on the moult or other debris, adhering slightly to the substratum and often collecting in groups.

During the first twenty-four hours the central blue substance changes into irregularly shaped refringent masses, evidently comparable to the 'plaquettes' described in other *Foettingeriidae* (fig. 4, Pl. 6). If a cyst is crushed at this stage, these exude as a fluid resembling oil, which does not mix with the surrounding water. The yellow granules which were enclosed by the blue mass in the active ciliate are still present in the

cyst at this stage, and also the granules of the surface protoplasm.

The refringent masses are gradually resolved into smaller and smaller fragments until they have the appearance of globules packed closely together, and this process is often accompanied by colour-changes. Some cysts become yellow, others remain blue, and some which were intensely blue at the beginning turn purple. Occasionally they have a reddish tinge. The yellow and blue cysts turn greenish in the last phase. After fixation the globules stain deeply with iron haematoxylin, and this becomes most marked in the later stages of schizogony. With Ehrlich's haematoxylin they only stain faintly. In most cases division begins some twenty-four hours after encystment, and the merozoites emerge on the third day about forty-eight hours after cyst-formation, but the time varies considerably. The smallest cysts hitherto noted have produced two merozoites and the largest ten; the number in a cyst may be uneven—four, five, and six young being produced in many instances (fig. 5, Pl. 6). (It is interesting to note that De Morgan in his paper on *Foettingeria* (1924) remarks: 'there is no apparent connexion between the size of *Foettingeria*, and its readiness to encyst and divide. Out of a sample of two or three dozen, ranging in length from about 250–500 μ , the expectation that one or all might encyst appears to be about equal.' He does not record the number of young produced, but they appear to have been numerous in every cyst.) The first sign of activity shown by the young is in the vibration of their cilia, and they soon afterwards begin moving round the inside of the cyst, creeping upon and over each other. This continues for about an hour and a half, the cyst wall being so thin and transparent that every detail can be seen. The contractile vacuoles are active during this entire period. The movements of the ciliates become extremely rapid before emergence, and when the cyst wall is ruptured they often escape in quick succession. In other cases some remain behind for a time, the wall being so elastic that it will close behind an escaping ciliate and appear unruptured. Great care must therefore be taken in counting the merozoites.

There is no micropyle, the first ciliate breaks through the cyst wall at any point. A cyst was once seen containing four ciliates, of which one burst out and two others fused within the cyst; these remained for another two hours revolving slowly, and then became violently active and emerged from the cyst and were lost sight of.

This is the only certain instance of syngamy hitherto observed within the cyst, and I am satisfied that it is not of general occurrence. There seems therefore little reason to regard these young forms generally as gametes, and for the present I shall continue to refer to them as merozoites.

It is just possible that the four merozoites above referred to were derived from two parent ciliates, for they were found in December, and encystment of double adult ciliates frequently takes place during the winter from November to April, and a few instances occur also in summer. Association takes place while they are still in the moult, and occurs both in large and small individuals and sometimes between two of greatly differing size. Individuals have been seen to join and remain revolving slowly inside the moult, the pairs emerging later, to swim actively about in the water with a jerky rolling motion. The plane of junction increases, and spiral twisting ensues, until the contractile vacuoles which were near the centre line appear near the opposite poles. Encystment follows in the same way as in single specimens, the nuclei being quite distinct at first. Fragmentation of the refringent substance proceeds in the normal way.

It does not appear that the cysts with double individuals produce more merozoites than large single cysts will do, but further investigation is much needed here and is difficult to carry out as material so often fails.

It is very noticeable that in autumn, before fusion begins, the average size of the ciliates is diminished, and they seem abnormally transparent and move feebly. No multiplication cysts have developed under observation in the laboratory at this time of year. Later, when double encystment has begun, it becomes possible to get both single and double cysts, and in

the spring the average size of the ciliates has greatly increased and schizogony in single individuals reaches its height during the months that follow.

Binary fission has never been seen to occur.

Robertson (1928) described encystment of double individuals of *Heteromita*, but this seems always to have been the result of delayed division, which is certainly not the case in *Physophaga*. On the other hand, the encystment of pairs in the latter has not so far been shown to represent any process of syngamy.

The Merozoites are 32μ to 40μ in length, ventrally flattened, and tapering slightly at the anterior end, where there is a small beak-like extension of clear protoplasm bent over to the right side, which is evidently the point of junction for the ciliary bands. The contractile vacuole is at the posterior extremity (fig. 6, Pl. 6). These merozoites are packed full of the substance already described in the multiplication cysts, no longer appearing in the form of globules but of solid refringent bodies, irregular in shape, greenish or yellowish in colour, occasionally retaining the purple tinge found in some cysts. After fixation these bodies stain with iron haematoxylin more deeply than the nucleus itself. The merozoites swim rapidly and strongly, revolving in the same way as the adults, or creep about on their ventral surfaces. The rosace is a very conspicuous feature at this stage, and it seems probable that it acts as a sucker in attaching the merozoites to their host. Hentschel (1927) recorded a new ciliate from the intestine of *Thalassema* and suggested that the mouth might be a kind of sucker, and this point is the more interesting since it has been shown that several of the Foettingeriidae are, like Hentschel's ciliate, internal parasites or commensals, besides having a second host on which the resting cysts are formed.

As the merozoites of Foettingeriidae do not ingest food, it may be that the rosace serves at this stage for purposes of attachment, and in the adult phase for purposes of suction and filtration as already suggested.

RESTING CYSTS.

Once when a freshly moulted *Gammarus* was put near the merozoites, they went to it and glided up and down the dorsal surface, apparently finding their way down the sides to the sterna and so avoiding the currents caused in the surrounding water by the action of the pleopods. They did not seem to go to *Gammarus* which had not recently moulted, but it has not been possible to confirm these experiments. The resting cysts are found at the base of the gills and appendages and along the articulations of the sterna, where the chitin is raised into ridges. In the angles of these ridges masses of resting cysts can often be found packed closely together. They are ovoid in shape, $27-32\mu$ long and $20-22\mu$ wide, and through the clear wall can be seen the yellow refringent bodies closely crowded together. The cysts are attached by a very short stalk, which is generally broad and wrinkled (fig. 7, Pl. 6). By the time the host is ready to moult, the ciliates within these cysts have become hyaline, the refringent contents have disappeared, and only some granules remain (which stain deeply with neutral red).

The first sign of activity within the resting cyst is the re-appearance and slow discharge of the contractile vacuole, after which ciliary action begins, and the ciliate bursts through the cyst wall. As soon as the moult takes place, these tiny ciliates become violently active, and assimilation and distension follow with extraordinary rapidity as already described.

METHODS.

The conditions that favour encystment and schizogony are very difficult to determine. When kept in large shallow pans in the laboratory the *Gammarus* retain their infection with these ciliates for months. It is therefore clear that the ciliates encyst and multiply in these pans after each moulting of the hosts.

During the spring and summer of 1926 and 1927 many cysts and large numbers of merozoites were obtained by leaving moults containing ciliates in small glass capsules where the water

could be renewed by means of two lengths of cotton wick. Even without such renewal the cysts often developed successfully, and they sometimes formed in only a few drops of water on cover-slips, so that they could be very conveniently fixed and also examined during development. This year, at the same season, hardly any specimens could be made to develop under similar conditions, though the infection in the pans continued. Experiments were tried at varying temperatures and by artificial aeration of the water, but without success.

It seems probable that only very healthy ciliates will go through the process of schizogony in the small vessels necessary for keeping them under observation, and the lack of strong specimens in some years may be accounted for by periodic depressions in the *Gammarus* stock itself, lack of vitality in the hosts possibly causing deficiency in the albumens normally eliminated with the moult, and consequently affecting the ciliates.

Unless specimens are in a healthy state, it is useless to attempt any close study of them. Moribund ciliates, such as often appear in autumn, burst so easily as to be almost worthless for examination. To get good material, the regular moulting of the *Gammarus* should be encouraged by often adding fresh water and feeding on dead elm leaves, alga, and wheat grains. Ciliates can be conveniently examined for minute structure in a drop of gelatine solution, spread into as thin a film as possible. In a hanging drop of this kind, movement is slowed gradually as evaporation goes on. Everything depends on getting the right consistency in the gelatine, and the ciliates will continue to revolve in a medium of surprising density, the action of the cilia being beautifully shown up.

Specimens taken out of a moult soon after it has been shed, and before assimilation is complete, will absorb ammonium carminate solution. When they have left the moult no absorption appears to take place.

With regard to fixation, nothing definite can be said, as the action of fixatives depends largely on the quantity and condition of the very peculiar metabolic substance. The difficulty

is to secure penetration of this internal mass without destroying surface structure. The best results obtained hitherto have been with films fixed in the mixtures of Carlton and Petrunckewitch—leaving the film in the fixative from forty minutes to two hours.

NATURE OF INCLUSIONS AND PIGMENTS.

Chatton, Lwoff, and Parat (1926) concluded that different species of decapods eliminate variously coloured carotinoid albumens at the time of moulting, and that these remain in the liquid of the moult and are ingested by the ciliates.

They remarked that *Polyspira*, found in moults of *Eupagurus prideauxii*, is usually dark violet in colour, but that specimens have been described by Minkiewicz from other decapods as being yellow, green, blue, or pink. As mentioned above, *Physophaga* in *Gammarus* moults is very occasionally bright yellow instead of blue. With regard to the normal blue colour, it may be worth noting that a dark blue-purple pigment is present in the eggs of the host. The oil globules in the fat-cells are generally bright orange.

The writers already quoted refer to the transformation of the pigmented substance which takes place in the multiplication cysts of *Polyspira* and *Gymnodinoides*. They regard it as a mass of fresh protein turning directly and in situ into a reserve material comparable to the vitellus of eggs. It is added that the colour-changes which take place in living cysts can be artificially induced by treatment with acids which coagulate or disintegrate the albumen, thereby separating it from the carotin.

In *Physophaga* the blue substance is turned rose colour by Schaudinn's fixative; Carleton's mercuric chloride and formalin mixture has been seen to precipitate red granules in specimens that had been dense and deeply blue.

In regard to the disappearance of reserve materials in the young ciliates before emerging from their resting cysts, there is little doubt that these materials are digested and that red granules are precipitated after the albuminous reserves have

been utilized. The carotinoid pigment therefore appears to be absorbed owing to its combination with the proteid and to be separated when the latter is digested, being apparently of no use to the ciliates.

NUCLEAR CHANGES DURING ENCYSTMENT.

Investigation of nuclear stages is difficult, as these are obscured by the reserve materials in the cyst. The micronucleus has not yet been observed in division, but I hope that further investigation may be carried on when material becomes more plentiful.

Remarkable changes occur in the macronucleus during the first stage of encystment, while the micronucleus is still in the resting state. Preparations stained with iron haematoxylin show the macronucleus to be finely granular, the granules sometimes appearing in lines. There is a central fusiform body which often projects at both ends of the nucleus, and seems to be composed of a bundle of darkly staining threads (fig. 8, A, Pl. 6).

Later on, the chromatin granules clump together to form irregular masses at the periphery of the nucleus, the fusiform central body persisting as before (fig. 8, B, Pl. 6).

In resting cysts of *Physophaga* the fusiform body again appears in the macronucleus. It stains deeply with iron haematoxylin but does not stain at all with carmine.

Delphy (1927) records an apparently similar appearance in macronuclei of 'les Anoplophryimorphes'.

Minkiewicz (1913) observes that in the schizogony of *Gymnodinoides* and *Polyspira* the chromatin of the macronucleus forms balls, threads, rods, &c.

SYSTEMATIC.

The family Foettingeriidae (Chatton) has hitherto consisted of six genera, all characterized by a life-cycle of at least three phases: (1) resting cysts on a crustacean host; (2) a phase of assimilation and vegetative development in the corpse or moult of the host; (3) a multiplication phase, resulting in merozoites

(called 'tomites' by the originator of this classification). Also by the possession of a 'rosace'. In the genera *Pericaryon*, *Spirophrya*, and *Foettingeria* the resting cysts open when the crustacean host is ingested by a Ctenophore, a Hydroid, or an Anemone respectively. *Synophrya* develops in the crustacean moult and goes through a fourth phase in the life-cycle, encysting within the host's integument.

Polyspira develops in the host's moult and then multiplies, unencysted, outside.

Gymnodinoides, after development in the moult, forms cysts possessing a micropyle. It has a dendriform macronucleus.

The distinguishing characteristics of the new genus *Physophaga* are: vegetative development in the crustacean moult followed by reproduction in a cyst outside; cysts without micropyle; macronucleus elliptical.

Physophaga sappheira is the first recorded instance of a freshwater form in the *Foettingeriidae*.

In conclusion I wish to express my best thanks to Professor Goodrich for the privilege of being allowed to work in the Department, and to Dr. Helen Goodrich for her constant help and kindness.

DEPARTMENT OF ZOOLOGY AND COMPARATIVE ANATOMY,
UNIVERSITY MUSEUM, OXFORD.

REFERENCES.

- Caullery, M., and Mesnil, F. (1903).—"Sur la structure nucléaire d'un infusoire parasite des Actinies, *Foettingeria* n.g. actiniarum, Clap.", 'C. R. Soc. Biol. Paris', vol. lv, p. 806.
- Chatton, E. (1911).—"Ciliés parasites des Cestes et des Pyrosomes, *Perikaryon Cesticola*", 'Arch. de Zool. Expér.', 5th series, Notes et Revue, VIII, p. 8.
- Chatton, E., and Lwoff, A. (1924).—"Sur un Infusoire marin, *Spirophrya subparasitica* n.g. n.sp., à deux hôtes, Copépode et Hydriare", 'C. R. Acad. Sci. Paris', vol. clxxviii, p. 1642.
- (1925).—"L'éthologie et la structure des *Spirophrya*; leur parenté avec les *Foettingeria*. L'origine et l'évolution du parasitisme de ces Infusoires", *ibid.*, vol. clxxx, p. 229.
- (1925).—"Sur la détermination physiologique des phases du cycle de l'*Infusoire Spirophrya subparasitica*", *ibid.*, p. 338.

- Chatton, E., and Lwoff, A. (1926 a).—"La structure et le cycle évolutif des Infusoires des mues de Crustacés, et leur place parmi les Foettingeriidae", *ibid.*, vol. clxxxii, p. 100.
- (1926 b).—"Infusoires parasites internes des Crabes. Leur évolution à la mue. Leur place parmi les Foettingeriidae", *ibid.*, vol. clxxxiii, p. 1131.
- (1927 a).—"Foettingeria actiniarum", *ibid.*, vol. clxxxv, p. 675.
- (1927 b).—"Les Métamorphoses des Foettingeriidae", *ibid.*, p. 1075.
- Chatton, E., Lwoff, A., and Parat, M. (1926 c).—"L'origine, la nature, et l'évolution du pigment des Spirophrya, des Polyspira et des Gymnodinoïdes", 'C. R. Soc. Biol. Paris', vol. xciv, p. 567.
- (1927).—"La formation, la nature, et l'évolution des réserves chez les Spirophrya, les Polyspira et les Gymnodinoïdes", *ibid.*, Séance Jan. 8.
- Claparède (1863).—"Plagiotoma actiniarum", 'Beob. Anat. d. wirbellosen Thiere, etc.', p. 2.
- Delphy, Jean (1927).—"Sur la constitution de l'appareil nucléaire chez les Infusoires : les Anoplophryimorphes", 'C. R. Acad. Sci. Paris', p. 1323.
- Foettinger, A. (1881).—"Infusoires de Céphalopodes, Opalinopsis", 'Arch. de Biol.', vol. ii, p. 367, pls. xxi, xxii.
- Hentschel, C. C. (1927).—"On a new ciliate, Ptyssostoma thalassemae, from the intestine of *Thalassema neptuni*", 'Journ. Marine Biol. Ass.', vol. xiv, no. 3, March, p. 651.
- Jourdan (1880).—"Les Zoanthaires du Golfe de Marseille", 'Ann. Sci. Nat., Zool.', 6^e série, vol. x, p. 77.
- Minkiewicz, R. (1912).—"Ciliata chromatophora", 'C. R. Acad. Sci. Paris', vol. clv, p. 513.
- "Un cas de reproduction extraordinaire chez *Polyspira Delagei*", *ibid.*, p. 733.
- (1913).—"Études sur les Infusoires syndesmogames", 'Bull. Internat. Acad. Sci. Cracovie', Class B, p. 742.
- Morgan, W. de (1924).—"Foettingeria actiniarum", 'Quart. Journ. Micr. Sci.', vol. 68, p. 343.
- Robertson, Muriel (1928).—"Notes on Heteromita, Protozoa Flagellata", 'Parasitol.', vol. xx, p. 13.

EXPLANATION OF PLATE 6.

Illustrating Lady Muriel Percy's paper on *Physophaga sappheira*, n.g., n.sp.

REFERENCE LETTERS.

a, aperture of contractile vacuole; *c.c.*, lines of cilia at margins of groove; *c.v.*, contractile vacuole; *f*, fragments of reserve substance; *g*, groove; *m*, mouth; *ma*, macronucleus; *mi*, micronucleus; *p*, anterior prominence; *r*, rosace; *s*, strands of protoplasm.

Fig. 1.—Ventral view of *Physophaga sappheira*. $\times 1,500$ approx.

Fig. 2.—Plan of ciliary bands, dorsal view.

Fig. 3.—Diagram much enlarged of mouth and rosace from ventral surface.

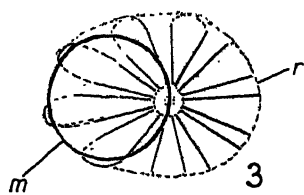
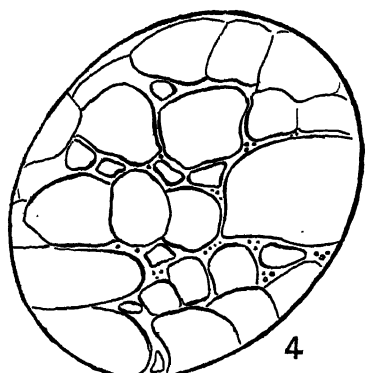
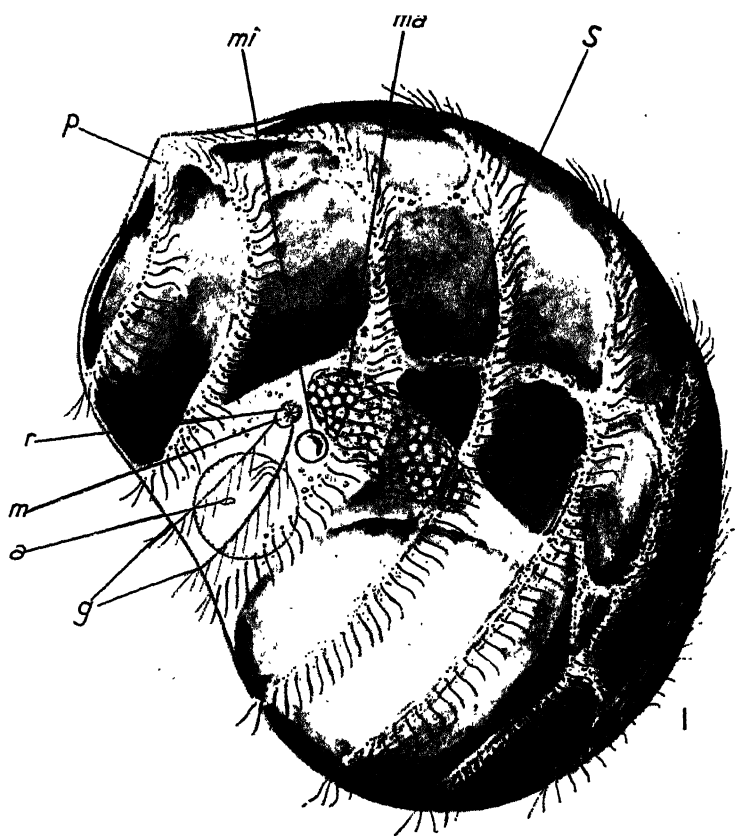
Fig. 4.—Multiplication cyst showing early fragmentation of the blue substance. $\times 1,200$.

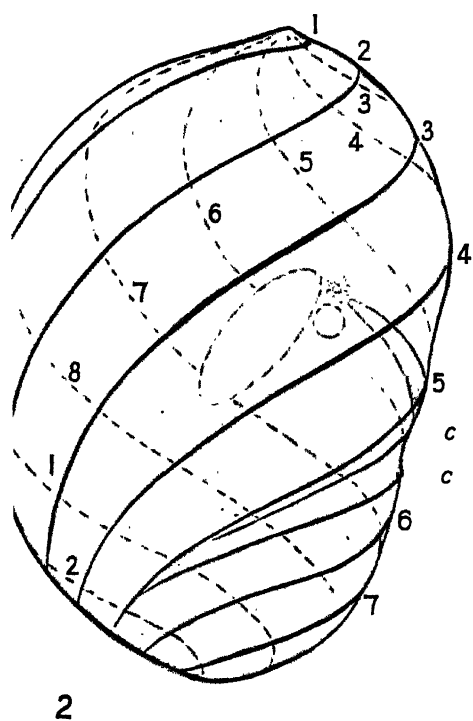
Fig. 5.—Multiplication cyst at later stage, containing six merozoites. $\times 1,200$.

Fig. 6.—Merozoite, ventral view. $\times 1,200$.

Fig. 7.—Resting cyst. $\times 1,400$.

Fig. 8.—Macronucleus in multiplication cyst, showing fusiform central body. $\times 1,400$. A, early stage. B, later stage.



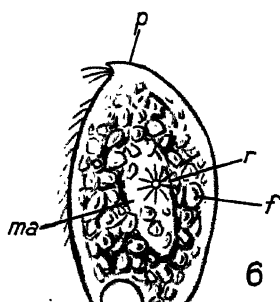
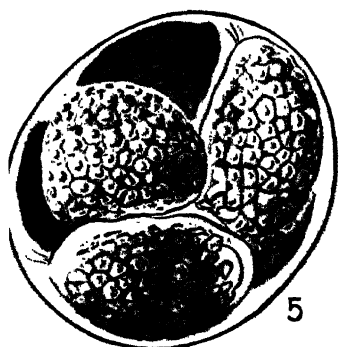
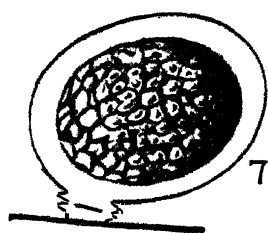


A



8

B



Lipin Secretion in the Elasmobranch Interrenal.

By

A. H. H. Fraser, M.B., Ch.B., B.Sc.

From the Plymouth Marine Laboratory.

With Plates 7, 8.

INTRODUCTION.

RETZIUS (1) in 1819 first described the suprarenals of fishes. He noted the resemblance between the cortical tissue of Elasmobranchs and the suprarenals of birds. Stannius (2) in 1846 described the suprarenals of the higher cartilaginous and bony fishes. He was aware only of the cortical tissue in Elasmobranchs. Balfour (3) in 1878 gave the first complete account of the Elasmobranch cortical tissue. He introduced the term 'interrenal' to refer to the unpaired body of Scyllium. The anatomy of the interrenal in different species was fully and accurately described by Swale Vincent (4) in 1897.

In previous work the histology of the organ has shed no light on its functional activity. This is because the resting condition of the gland is that most commonly met with, and consequently this particular phase of glandular activity has been erroneously described as its constant histological appearance. It has long been known that the Elasmobranch interrenal in common with its homologues in higher vertebrates contains an unusual concentration of lipin in its cells. These lipins are readily demonstrable by all the usual histo-chemical tests. In an organ composed of glandular epithelial cells the presence of an exceptionally large amount of lipin naturally suggests the possibility of its being a secretory product. Since the interrenal is a ductless gland, such a secretion, provided it occurs, must take place into the efferent blood-vessels of the organ.

Such a process of secretion in the suprarenal of the guinea-pig

was described by Mulon (5) in 1902, but his work appears to have been largely ignored. Watrin (6) in 1924 described similar appearances in guinea-pigs, following inoculation of their suprarrenals with *B. coli* culture. There appears to be no description of lipin secretion in the interrenal of Elasmobranchs.

The present paper describes the results of an intensive histological study of the interrenal of about thirty rays (*Raja clavata*). It has been found that while the majority of glands correspond to the usual description of an 'ochre-yellow' body, with the lipin confined to its constituent cells, a minority (about one in ten) show a brown coloration of varying intensity. When sectioned, these brown glands show evidence of active lipin secretion, in some cases the greatest concentration of lipin being in the interlobular capillaries. The process of secretion is peculiar, and appears to be somewhat analogous to the secretion of milk by the mammary gland; with the fundamental difference that while the milk passes into lactiferous ducts the lipin of the interrenal passes into the circulation.

METHODS.

Raja clavata was the only species investigated. At first fish of all ages and sizes were used, being selected at random from the catches brought in by the laboratory boat. Later, large mature fish were employed, since it was found that among these the proportion of actively secreting glands was greater.

The fish were killed by destruction of the brain, and immediately dissected. The dorsal route was found to be the best method of access to the interrenal. One longitudinal incision parallel and close to the vertebral column is sufficient to give access to the interrenals of both sides. The incision is carried down to the kidney through the dorsal musculature and fascia, the vertebral column being drawn over to the opposite side. By this method complete exposure of the interrenal is secured without division of the peritoneum.

The interrenal was removed by slicing off the underlying portion of kidney tissue. No attempt at dissection was made, in order to save time and avoid damage to the interrenal. The

tissue was momentarily rinsed in normal saline and then placed in the appropriate fixative. The interrenal with subjacent kidney tissue was removed in four different portions, and each portion fixed by a different method. The fixatives employed were :

- (1) Bouin's picro-formol-acetic.
- (2) Ciaccio's chrome-formol-acetic with post-chromation.
- (3) Muller's fluid with post-osmication by Marchi's method.
- (4) Five per cent. formol.

Latterly Schridde's post-osmication method was also used.

Bouin's method was employed to demonstrate general histological structure and the distribution of pigments insoluble in fat solvents.

Ciaccio's method, which had been found useful in the rabbit, proved disappointing in the ray. The sections stained faintly and diffusely with Sudan III, and no clear differentiation of lipin could be obtained.

Marchi's method gave the clearest picture of lipin distribution. Figs. 3 to 11 inclusive are taken from sections prepared by this method.

Formol-fixed tissue was sectioned on the freezing microtome. Sections were used to test the reactions and solubilities of the pigments and for examination under the polarizing microscope. Sections were stained with 1 per cent. aqueous solution of osmic, Sudan III in 70 per cent. alcohol, and 1 per cent. aqueous solution of Nile blue. These stains were also used for staining sections previously soaked in fat solvents.

Practically the entire 'complete histochemical investigation of fatty-cell inclusions' recommended by Cramer and Gatenby (7) was therefore performed. While the results have given useful information regarding the distribution of lipin in the gland, the evidence as to the nature of the lipins present remains inconclusive.

This was in part anticipated from the fact admitted by Cramer and Gatenby, that the reaction of the various lipins to these histochemical tests is entirely modified when different lipins are present in the same globule. Unfortunately that is the

condition in which lipins occur in the interrenal, and probably in most other organs of the body.

In any case it is known that the physical condition of the lipins completely modifies their staining reactions to such an extent that they may become completely undetectable by histological methods (Bloor (8); Carleton (9)).

Nevertheless, the histological method is of value in demonstrating variations in the nature and distribution of lipins in different individuals. The information regarding distribution is of supreme value, for it is information that present methods of chemical analysis fail to give.

RESULTS.

1. Relation of Interrenal and Chromaffin Tissues.

While in the ray the interrenal and chromaffin tissues are usually separated by intervening kidney tissue, this is not invariably the case. Fig. 1, Pl. 7, shows how close the anatomical relation may be. The chromaffin body lies in the concavity of the interrenal, like a stone in the hollow of a hand. A thin layer of connective tissue alone separates the two tissues. There is, however, no intermingling of strands as in Amphibia, Reptiles, and Birds. The figure illustrates a fact which has been already shown experimentally by Swale Vincent (10). In chemical work on the interrenal the possible contamination by chromaffin tissue cannot be ignored. Fig. 2, Pl. 7, is a higher magnification of the boundary between the two tissues.

2. The Secretion of Lipin.

(a) The Resting Gland.—In nine out of ten glands selected at random during the months of May, June, and July the interrenal varies in colour from a creamy white to a bright yellow. That this colour is due to a lipochrome pigment is shown by its solubility in acetone and alcohol. The variability in brightness of lipochrome pigmentation may be related to food. Findlay (11) showed that the lipochrome of the fowl's adrenal is directly derived from the food. Similarly the lipochrome of the

ray's interrenal may be derived from the crustacea which are almost constantly found in its stomach.

The lipochrome is completely dissolved by treatment by Bouin's method, and no pigment insoluble in fat solvents can be detected.

Fig. 3, Pl. 7, is a Marchi section from such a yellow gland. It was taken from a young immature male. The lipin is stained black by osmic acid. It is immediately obvious that the quantity of osmicated lipin in the interrenal vastly exceeds that in the adjacent kidney tissue.

The gland is seen to be surrounded by a firm connective-tissue capsule, and to be broken up into a large number of lobules, cut in various planes. Blood-vessels run between the lobules, so that blood separates each lobule from its neighbours. Serial sections show the lobules to be sections of cell columns running in all directions. It is doubtful whether these columns contain a central space in the resting condition. Such an appearance is common, but might readily be an artifact. But in Bouin sections strongly stained with eosin and differentiated, blood corpuscles can be observed in the centre of the columns. It is difficult to conceive how this could be an artifact.

In transverse section the columns measure 30–40 μ . The osmicated lipin is confined to the cells; none is visible between the columns. The lipin is diffusely distributed throughout the cells, but is more concentrated at the periphery of the columns.

This is the commonest histological picture encountered. The yellow gland, with lipin confined to constituent cells, and with no evidence of cellular hypertrophy, is assumed to be in the resting inactive phase. From this picture the phases of active secretion can be readily derived, although differing so much on superficial examination as to suggest a different organ.

(b) The Hypertrophied Lobule.—One out of about ten glands shows a brown pigmentation in varying degrees of intensity. Fig. 4, Pl. 7, is a Marchi section of a definitely brown gland, taken from a large female with ripe ova. The lobules are clearly immensely hypertrophied. They average 160 μ in diameter. The individual cells measure 80 μ . The degree of

hypertrophy of the lobule is shown by comparison with the lobules of the resting gland (cf. fig. 8, Pl. 7). The hypertrophied lobule is four to five times larger than that of the resting gland, and the individual cells of the hypertrophied lobule exceed the entire resting lobule in size. An examination of the individual lobule in fig. 4, Pl. 7, shows two important facts :

- (1) The cells are in various stages of disintegration.
- (2) The lipin is concentrated in large masses. This concentration of osmicated lipin in the cells does not necessarily mean that the lipin existed in such large globules during life. What can confidently be stated is that the quantity of lipin in the cells of the hypertrophied lobule is much greater than that of the resting lobule.

The most probable interpretation of this cellular hypertrophy appears to be as follows. In a secreting cell where the cell membrane is permeable to the products of secretion, no great enlargement of the cell is possible, since the secretory products are carried away either into ducts or blood-vessels. But suppose the cell membrane impermeable to the secretory product, then the secretion must accumulate in the cell, the cell membrane undergoing progressive distention and final rupture. On this interpretation the hypertrophied lobule is in a phase of intracellular secretion, and the partial disintegration of the cells becomes a necessary corollary.

(c) Formation of Acini.—Fig. 5, Pl. 7, is a Marchi section from the same gland as fig. 4, Pl. 7. It shows the final result of lobular hypertrophy and disintegration. Disintegration of the cells in the centre of the lobule evidently leads to the development of an acinus, down which the products of secretion can pass. The distended connective-tissue capsule of the lobule becomes the wall of the acinus. In fig. 5, Pl. 7, both acini and distended lobules are visible in the same field. It requires little imagination to understand how one is derived from the other. By progressive distention of the resting lobule an hypertrophied lobule is formed, which in turn forms an acinus by cellular disintegration. It is noteworthy that the acini figured show

evidence of lipin-containing cytoplasm and of pale nuclei around their walls. It seems probable from this and other sections that the cellular disintegration is incomplete.

Fig. 6, Pl. 7, is a Marchi section of a light-brown gland taken from a large pregnant female. It shows two well-marked acini separated by an interlobular capillary. Two collapsed acini can also be distinguished, one in the angle between those still distended. By collapse of the distended acini and growth of the basal parts of the cells left on cellular disintegration the resting lobule is reformed.

Fig. 7, Pl. 7, shows another acinus from a different part of the same gland from which fig. 6, Pl. 7, is taken.

The interrenal tissue therefore appears to undergo a definite cycle of changes during active secretion. These are :

- (1) The resting lobule.
- (2) Distention of lobule.
- (3) Disintegration of lobule.
- (4) Formation of acinus.
- (5) Collapse of acinus.
- (6) Reformation of resting lobule.

Assuming the correctness of this interpretation of the observed data, what becomes of the secreted lipin after its passage down the acinus? Since the interrenal is a ductless gland it must obviously pass into the blood, and should be demonstrable in the capillary network of the organ.

Fig. 8, Pl. 7, is a Marchi section of a dark-brown gland taken from a large ripe male. Here the lipin is concentrated in the interlobular capillaries. The network of capillaries shown up by their black contents gives an appearance of artificial injection. The lobules on transverse section measure 40μ . Apparently the acini have collapsed to form lobules, and the lipin is passing through the sinuses of the organ to reach the general circulation.

The histologically demonstrable presence of lipin in the blood-spaces of the gland is of sufficient importance to warrant special discussion. Three possible interpretations suggest themselves.

- (1) That the appearances are artifacts. Now, where under a

special histological technique a certain structure or appearance is constantly obtained, the possibility cannot be ignored that the structure demonstrated may in reality be an artifact produced by the method employed. But where, under a precisely similar technique, such diverse appearances as figs. 3 and 8 are obtained, the presumption is that the appearances demonstrated represent different physiological conditions of the organ. In any case it is difficult to conceive how the action of fixatives could produce an appearance of fat-choked capillaries.

(2) That the lipin is in process of transport from the blood to the interrenal cells. It is inconceivable that lipin could accumulate to such an extent in the general circulation without assuming a generalized lipaemia. Now a lipaemia of such extent would almost certainly be incompatible with life. Further, no evidence of a lipaemia is detectable in the adjacent kidney tissue.

(3) The only feasible explanation appears to be, that lipin has passed from the interrenal cells into the circulation. Since the outstanding histological feature of the interrenal is its large lipin content it seems conceivable that its cells could secrete lipin in sufficient quantity to fill the capillaries of the organ. It appears justifiable to assume that the lipin in the capillaries is the secretion of the interrenal cells, and that the gland has been fixed in process of active secretion.

Fig. 9, Pl. 8, is a higher magnification of part of fig. 8. It has been focused to show the network of lipin-filled capillaries.

Fig. 10, Pl. 8, is also a higher magnification of fig. 8, but is focused to show the lobules. It is seen that while the lobules contain a certain amount of diffusely distributed lipin, the main concentration of lipin is in the interlobular capillaries between the lobules.

Fig. 11, Pl. 8, is a Marchi section from a yellow gland. Part of the same gland fixed by Bouin's method showed a small amount of brown pigment, evidently rendered visible by solution of the masking lipochrome. The lobules measure 30μ on transverse section. They are closely packed together (cf. fig. 3, Pl. 7) and traces of lipin can be observed in the capillary net-

work. This is presumably a later stage of secretion than fig. 8, Pl. 7, and leads us back to the resting gland first described.

Fig. 12, Pl. 8, is a Ciaccio section taken from the same dark-brown gland of which fig. 8, Pl. 7, is a Marchi preparation. It shows dark-brown intracellular pigment granules. This pigment occurs in about one in ten glands, and is always associated with appearances of lipin secretion in the series examined. The two glands which showed the greatest density of brown pigmentation were those two which also showed a concentration of lipin in the interlobular capillaries. The pigment can be demonstrated microscopically without staining, after fixation by the methods of Bouin and Ciaccio.

In formol-fixed sections the pigment persists. In these it was found to be insoluble in water, acetone, alcohol, and ether; to bleach rapidly with H_2O_2 and less rapidly with 5 per cent. NaOH. Since it persisted after Bouin fixation, it follows that it is insoluble in dilute acids. From its solubilities and bleaching reactions it may be assumed that the pigment belongs to the melanin group.

3. The Chemical Nature of the Secretion.

The histochemical tests employed failed to yield any definite indication of the nature of the lipin, either in the lobules of the resting gland or in the capillaries of the secreting organ. The demonstration of the different phases of glandular activity is a question of histology, suitable to histological investigation. Histology also shows that the substance secreted is of a lipin nature. But the exact composition of the secretion, whether neutral fat, cholesterol, phosphatide, or a mixture of these, is a biochemical problem demanding chemical methods of attack. Speculation on this question is therefore useless in the absence of definite chemical data.

DISCUSSION.

It is advisable at the outset to separate the definitely ascertained facts from their hypothetical interpretation.

In a series of interrenals subjected to an identical histological

technique a marked variation in structure and lipin distribution was clearly displayed. The presence of lobules, hypertrophied lobules, acini, and lipin-choked capillaries may be taken as proved. The possibility of artifact can reasonably be excluded. But the interpretation of these structures as phases in a definite cycle of glandular activity is necessarily hypothetical. It is, however, an interpretation which fits the facts. It is nevertheless possible that the different phases of glandular activity have been placed in a false-time relationship. It is conceivable, for example, that the stage of fat-choked capillaries precedes that of the hypertrophied lobule ; conceivable, but improbable.

The point of fundamental importance is, of course, the demonstration of lipin in the capillaries. It affords a very valuable clue to the function of the interrenal.

The occurrence of melanin pigment in glands shown to be in a secretory phase, and its absence in glands where no evidence of secretion could be detected, is probably significant. In the first twelve glands examined only one was brown, and this brown gland was the only one to show evidence of secretion. In subsequent work a deliberate search for brown glands was made, on the working hypothesis that these would also show evidence of secretion. This prediction was fully borne out in the results. In twenty yellow glands examined histologically there was no evidence of secretion. The six brown glands examined by the same methods all showed evidence of secretion.

The conclusion seems justified that the formation of melanin and the secretion of lipin in the gland have a definite metabolic relation.

A question arises as to the fate of the melanin. How is it disposed of ? Does it, like the lipin, find its way into the circulation ? It is possible under high magnification to demonstrate the presence of pigment granules in the capillaries of secreting glands. But this is never so definite as the evidence of lipin secretion, and is not sufficiently diagrammatic to repay reproduction.

The nature and function of the lipin secretion, the fate and mode of formation of the melanin pigment, the relation be-

tween these two sets of phenomena, are fascinating subjects for speculation. The preliminary work done on these subjects, however, has not yet yielded any definite conclusions, and the matter remains too hypothetical to justify discussion.

SUMMARY.

1. The occasional occurrence of an intimate anatomical relationship between the interrenal and chromaffin tissue is described.

2. It is shown that in addition to the ordinary lobule commonly figured, the interrenal may show hypertrophied lobules, disintegrating lobules, and acini. These various histological pictures are interpreted as being various phases in a definite cycle of glandular activity, viz. :

- (1) Resting lobule.
- (2) Distension of lobule.
- (3) Disintegration of lobule.
- (4) Formation of acinus.
- (5) Collapse of acinus.
- (6) Reformation of resting lobule.

3. Evidence is advanced of a massive secretion of lipin into the interlobular capillaries. The importance of this phenomenon is emphasized.

4. The occurrence of melanin pigment in a minority of the glands is described, and evidence submitted of a relationship between the formation of melanin and the secretion of lipin.

ACKNOWLEDGEMENTS.

I desire to thank the British Association and Mr. T. H. Riches for the use of their tables at the Plymouth Marine Laboratory. I should also like to express my gratitude to the staff there, and particularly Mr. Pantin, for their courteous assistance and encouragement.

REFERENCES.

1. Retzius.—'Observat in Anat. Chondropteryg.' Lund, 1819.
2. Stannius.—In Muller's 'Archiv.', 1839, 3, 97 ff.
3. Balfour.—'A Monograph on the Development of Elasmobranch Fishes.' London, 1878.

4. Swale Vincent.—“Suprarenal Bodies in Fishes and their relation to the Head-kidney”, ‘Trans. Zool. Soc.’, London, 1898, 14, 41.
5. Mulon.—“Excrétion des capsules surrénales du cobaye dans les vaisseaux sanguins”, ‘C. R. Soc. Biol.’, 1902, 54, 1540.
6. Watrin.—“Réaction pigmentaire expérimentale des capsules surrénales”, *ibid.*, 1924, 90, 1061.
7. Cramer and Gatenby.—‘The Microtomist’s Vade-Mecum.’ London, 1928.
8. Bloor.—“Fat Transport in the Animal Body”, ‘Physiol. Reviews’ : 1922, 2, 92.
9. Carleton.—‘Histological Technique.’ Oxford, 1926.
10. Swale Vincent.—‘Internal Secretion and the Ductless Glands.’ London, 1924.
11. Findlay.—“The Pigments of the Adrenals”, ‘Journ. Path. and Bact.’, 1920, 23, 482.

EXPLANATION OF PLATES 7 AND 8.

All the figures are taken from sections of the interrenal body of *Raja clavata*.

LIST OF ABBREVIATIONS.

ac., acinus; *ac.c.*, acinar cavity; *c.*, capsule; *chr.t.*, chromaffin tissue; *cp.ac.*, collapsed acinus; *c.t.b.*, connective-tissue boundary; *cyt.*, cytoplasm; *i.l.c.*, interlobular capillaries; *ir.t.*, interrenal tissue; *k.t.*, kidney tissue; *lb.*, lobule; *n.*, nucleus; *os.lip.*, osmicated lipin; *pig.gr.*, pigment granules; *pr.dis.*, products of cellular disintegration.

PLATE 7.

Fig. 1.—Photograph, untouched. Magnification $\times 80$. Transverse section of interrenal and chromaffin bodies demonstrating the occasional occurrence of an intimate anatomical relationship between these two tissues.

Fig. 2.—Photograph, untouched. Magnification $\times 360$. A higher magnification of fig. 1 to show the presence of a connective-tissue boundary between the interrenal and chromaffin tissues.

Fig. 3.—Photograph, untouched. Magnification $\times 80$. Transverse section of a yellow, resting gland, showing the small size of the individual lobules and the osmicated lipin confined to the lobular cells.

Fig. 4.—Photograph, untouched. Magnification $\times 80$. Transverse section of a brown, active gland, showing the large size of the individual lobules and black masses of osmicated lipin in the lobular cells.

Fig. 5.—Photograph, untouched. Magnification $\times 80$. Transverse section of a brown, active gland, showing disintegration of the hypertrophied lobules to form acini.

Fig. 6.—Photograph, untouched. Magnification $\times 80$. Transverse section of a brown, active gland, showing fully formed acini containing the products of cellular disintegration.

Fig. 7.—Photograph, untouched. Magnification $\times 80$. Transverse section of light brown, active gland, showing a fully formed acinus.

Fig. 8.—Photograph, untouched. Magnification $\times 80$. Transverse section of dark brown, active gland, showing the concentration of osmicated lipin in the interlobular capillaries.

PLATE 8.

Fig. 9.—Photograph, untouched. Magnification $\times 360$. A higher magnification of fig. 8, focused to show the osmicated lipin in the capillary network.

Fig. 10.—Photograph, untouched. Magnification $\times 360$. A higher magnification of fig. 8, focused to show that the greater concentration of osmicated lipin is between the lobules in the interlobular capillaries.

Fig. 11.—Photograph, untouched. Magnification $\times 80$. Transverse section of a yellow gland, showing traces of osmicated lipin in the capillary network and the lobules closely packed together.

Fig. 12.—Photograph, untouched. Magnification $\times 360$. Transverse section of dark brown, active gland, showing the presence of melanin pigment in the lobular cells.

Fig. 13.—Drawing, semi-diagrammatic. From a transverse section of a yellow, resting gland, showing four lobules separated by the interlobular capillaries.

Fig. 14.—Drawing, semi-diagrammatic. From a transverse section of a brown, active gland, showing an individual hypertrophied lobule.

Fig. 15.—Drawing, semi-diagrammatic. From a transverse section of a brown, active gland, showing the disintegration of an hypertrophied lobule.

Fig. 16.—Drawing, semi-diagrammatic. From a transverse section of a brown active gland, showing an acinus formed by the disintegration of an hypertrophied lobule.

Fig. 17.—Drawing, semi-diagrammatic. From a transverse section of a brown active gland, showing that the lobular disintegration is not complete, a margin of cellular tissue being retained.

Fig. 18.—Drawing, semi-diagrammatic. From a transverse section of a brown active gland, showing the collapse of an acinus to reform the resting lobule.

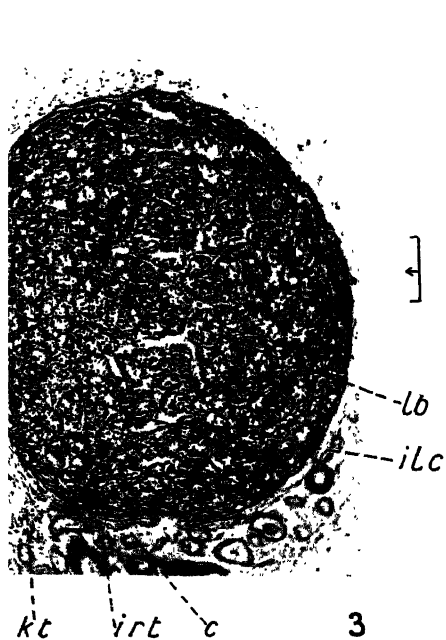
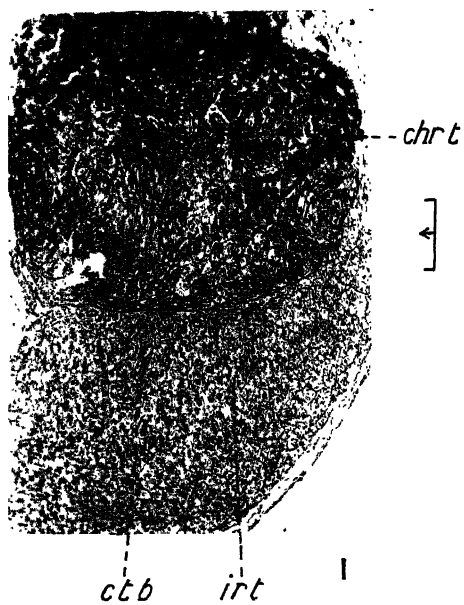
Fig. 19.—Drawing, semi-diagrammatic. From a transverse section of a dark brown, active gland, showing the presence of osmicated lipin in the interlobular capillaries.

Fig. 20.—Drawing, semi-diagrammatic. From a transverse section of a yellow, resting gland, showing a compact lobular arrangement and traces of osmicated lipin in the interlobular capillaries.

Fig. 21.—Drawing, semi-diagrammatic. Three cells of a yellow, resting gland, showing the absence of pigment granules.

Fig. 22.—Drawing, semi-diagrammatic. Three cells of a dark brown, active gland, showing the presence of melanin pigment granules in the cytoplasm.







5



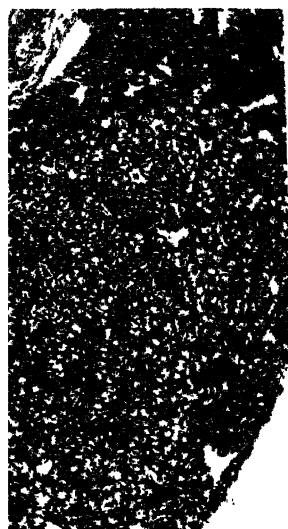
6

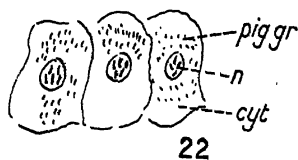
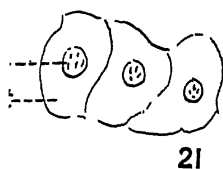
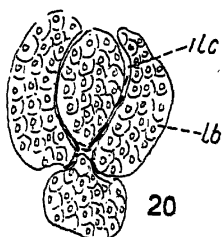
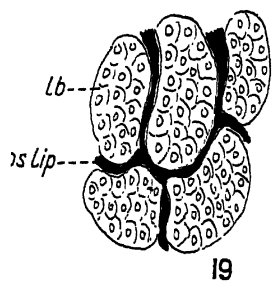
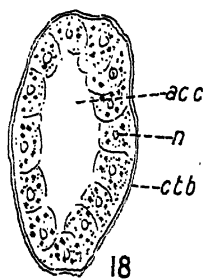
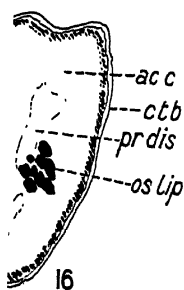
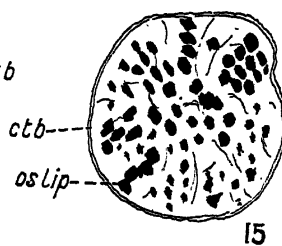
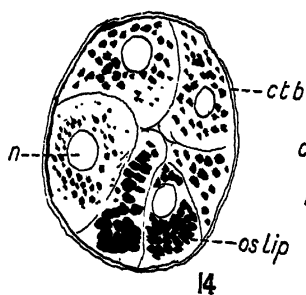
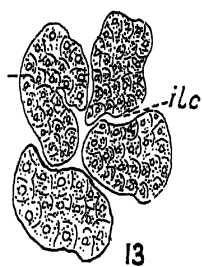


7



8





Mitochondrial Behaviour during the Life-cycle of a Sporozoon (*Monocystis*).

By

E. S. Horning, D.Sc.

Department of Zoology, University of Melbourne.

With Plate 9.

INTRODUCTION.

At the present time the behaviour and function of mitochondria within the animal and plant cell is a matter of controversy among cytologists, and, in order to help to elucidate the nature of these bodies, observations were made upon these cytoplasmic inclusions within a common sporozoon (*Monocystis*). The changes which mitochondria undergo during the life-cycle of this organism, together with the role they appear to play in cellular metabolism, is also discussed.

LIFE-CYCLE OF MONOCYSTIS.

If the sperm-sacs of the common Australian (European) earthworm are examined, it will be noticed that they are occasionally infected with *Monocystis*. The life-cycle of the parasite is well known. When reproduction is about to take place the two trophozoites or adult individuals come together, become rounded off, and finally secrete a cyst, in which two gametocytes become enclosed. The two nuclei of the adult gametocytes divide repeatedly until a large number of nuclei are formed. Later, each individual nucleus becomes surrounded by a small quantity of protoplasm. From this stage small minute cells (gametes) are finally formed. These minute gametes subsequently combine in pairs, and from these the zygotes or spores arise, so that the cyst at this phase of the life-cycle contains many such bodies. The nucleus of each zygote

undergoes fission giving rise to a number of fusiform sporozoites, generally eight in number. The spore coat finally ruptures and these young fusiform sporozoites are freed, and then make their way to the developing clumps of sperm, eventually becoming surrounded by sperm-cells. Finally, they grow into the adult organism.

METHODS OF INVESTIGATION.

In order to observe the behaviour of mitochondria during the life-cycle of this Sporozoon, the sperm-sacs of the common European earthworm, which occasionally swarm with *Monocystis*, were fixed in osmo-chromic fluid or else in a Champy solution. The fixed material was then washed in running water for a period of twenty-four hours, brought up through the alcohols and eventually embedded in paraffin, and sectioned. The sections were then cut $2-5\mu$ in thickness and stained with Heidenhain's iron haematoxylin, and occasionally counter-stained with eosin.

An examination of these sections with high magnifications shows that the cytoplasm of the trophozoite or adult individual contains numerous small, bent, rod-shaped bodies, together with a smaller number of spherical bodies scattered irregularly throughout the medullary region of the organism (see fig. 1, Pl. 9). These cytoplasmic inclusions certainly present a bacterial appearance, but their subsequent reaction to certain stains and fixing reagents shows quite clearly that they cannot be foreign organisms. Also, at various stages of the life-cycle, these bacteria-like bodies stained a bluish green when treated *intra vitam* with a Janus green B solution of about 1:10,000. It has been frequently shown by various authors that this *intra vitam* method does not appear to have any corresponding selective action upon bacteria, while Cowdry (1) and many other investigators have found that mitochondria in living cells stain specifically with Janus green B. Various other tests for mitochondria were also carried out, similar to those I have already described in some of my previous

papers (4, 5, 6, 7, 8, 9, 10), and the results show quite clearly that these protoplasmic bodies are true mitochondria reacting in the same manner as mitochondria present in other animal and plant cells.

MITOCHONDRIAL BEHAVIOUR DURING THE LIFE-CYCLE.

When the adult organism or trophozoite, cut in longitudinal section and stained with the Heidenhain process, is examined under high magnifications, the cytoplasm is found to contain a large number of bent rod-shaped and spherical mitochondria, scattered irregularly throughout the protoplasm of the medullary region of the parasite (see fig. 1, Pl. 9). A closer study of these mitochondria shows that they increase in number by means of transverse binary fission. A more detailed observation of the general arrangement of these bodies will reveal a dense aggregation of mitochondria in the vicinity of the nucleus (see fig. 1, Pl. 9) lying in close contact with the outer surface of the nuclear membrane. When dealing with this phenomenon in previous papers (9, 10), I have pointed out that it may possibly be a surface-tension effect which is apparently dependent upon the phosphatidal nature of the mitochondria. When stained sections of the next stage of the life-cycle—the early sexual phase—are examined, the cytoplasm of the encysted gametocytes is observed to contain numerous rounded mitochondria, often varying in size; while on the other hand the number of rod-shaped bodies, such as were seen in the previous stage, have undergone a considerable decrease in number (see fig. 2, Pl. 9). These larger rounded bodies probably arise through a fusion of several mitochondria. This apparent fusion of mitochondria has been observed by several authors, among whom is W. J. M. Scott (13), who describes a similar phenomenon occurring in the pancreas; and Cowdry believes that the larger spherical bodies are the result of a coalescence of several or more mitochondria. Later, I was able to demonstrate the actual fusion of mitochondria in living amoebae (7). This constant fusion of mitochondria during certain phases in many cells may possibly be explained in terms of surface-tension, and may be similar to the

fusion of oil-drops in suspensions of oil in water, and it does not seem likely that any physiological function can be attached to it. If sections showing the small gametes within the cyst are now examined, it will be noticed that the protoplasm of these minute cells contains small spherical mitochondrial bodies varying in size (see fig. 3).

During the true sexual phase of the life-cycle of the organism, when union of the gametes occurs, a noticeable fusion of mitochondria takes place (see fig. 3, Pl. 9), since the rounded mitochondria during this process tend to lose their spherical form and become irregular clumps. I have observed this effect on several occasions, and it is of interest to note that the same phenomenon occurs during the sexual phase in the binucleate *Opalina* (5) when conjugation of the gametes occurs.

The protoplasm of the two encysted gametocytes is not entirely used up to form the young gametes, and a surplus of residual cytoplasm is always left over and is termed by many authors the 'cystal residuum'.

An examination of this surplus cytoplasm within the cyst reveals the presence of several types of granules within it (see fig. 3, Pl. 9), mitochondria, lipoidal droplets, together with a third type of granule, which appears light blue after staining with Heidenhain's iron haematoxylin. These latter types of grains have the appearance of vegetative bodies. As the cyst becomes more mature these granules disappear as the residual cytoplasm becomes absorbed (see fig. 4, Pl. 9).

In order to ascertain the nature of these granules within the 'cystal residuum' the sperm-sacs of many earthworms infected with *Monocystis* were placed in a saline solution, teased, and examined for cysts. A portion of the sperm-sacs, containing several fairly large cysts, was removed on to a clean slide, and with the aid of needles the cyst walls were then ruptured. In order to test for mitochondria a Janus green solution of about 1:10,000 was introduced under the cover-slip containing the cyst. Several granules (the mitochondria) stained a bluish green, while other granules within the residual protoplasm, presumably the lipoidal and vegetative grains, did not show

a selective action to this stain. In order to ascertain the nature of the former type of granule a solution of Soudan III was run under the cover-slip containing another such ruptured cyst, and in this case the fatty droplets were observed to stain a light yellowish red, which demonstrated their lipoidal nature.

If the young zygote is examined in a fairly mature cyst the cytoplasm is seen to contain a variable number of rounded mitochondria. But later examination of the young spores, which are derived from the zygotes before the chitinous case is fully secreted around them, reveals that the mitochondria during this stage have undergone a notable decrease in number, and when finally the spore has reached maturity it is found to be entirely devoid of mitochondria. Sections cut through many such mature zysts show the complete disappearance of mitochondria both within the spores themselves and also within the cyst, and the 'cystal residuum' at this stage of the life-history of the parasite has become completely absorbed.

It cannot be suggested that this effect is due to a faulty fixation or technique, nor that the fixing fluids were unable to penetrate the chitinous case of the mature spores; because in every case the individual nuclei in each spore stained sharply and clearly, while the protoplasm of the sporozoite stained a palish blue when treated with the Heidenhain process.

In several cases material was fixed in a solution containing 4 per cent. osmic instead of the usual 2 per cent., and each time the same results were obtained. Material was also left in both Champy and osmo-chromic fixatives for a period of forty-eight hours, and even in over-stained cells no traces of mitochondria could be seen.

The next step in the life-history occurs when in another earth-worm the mature spore-coat finally ruptures and the young sporozoites within are liberated. They immediately become active and make their way to the developing sperm, where they gradually increase in size, and develop into the adult individual or trophozoite.

Stained sections of these young organisms on examination once more reveal the presence of small rounded and rod-shaped

mitochondria scattered irregularly throughout the medullary region of the cell. As the sporozoite gradually develops into the trophozoite the mitochondria increase in number. Continued observation of progressive stages of development has shown that the awakening of the cell is accompanied by a reformation and rapid reproduction of the mitochondria.

The behaviour of the mitochondria during the life-cycle of this organism, and especially their apparent origin *de novo* during chemical resynthesis, do not lend any support to Wallin's (14) contention that mitochondria are symbiotic organisms.

ON THE ROLE PLAYED BY MITOCHONDRIA DURING CELLULAR METABOLISM.

That mitochondria play an important part in the metabolism of the cell was first put forward by Guillermond (3), who suggested that starch and other plastids produced in plant cells owe their origin indirectly to mitochondria. Cowdry (2) also formulated the theory that these substances are originally formed within the mitochondria which later enlarge to form the body of the plastid, while Regaud (12) suggested that mitochondria are able to select certain materials from the surrounding protoplasm of the cell and build them up into various products. Later, while studying the behaviour of mitochondria within a common endoparasite protozoon—*Opalina* (5)—I was able to detect vegetative granules arising in close connexion with the surface of each single mitochondrion. These observations tend to support the speculation that mitochondria are the loci of protein and of general protoplasmic synthesis within the living cell. More recently the work of Marston (11) has suggested that the mitochondria contain concentrated within them the enzymes which bring about cell synthesis, their action being to build up protein at their surface. Moreover, the behaviour of mitochondria appears to illustrate the capacity of enzymes to reverse their activity in accordance with conditions, i.e. synthesis or hydrolysis, according to the concentration of the substrate. For example, in *Opalina* we see that mitochondria

synthesize vegetative products at their surface; while in *Paramoecium* (6), *Nyctotherus* (8), and *Amoeba* (7) the function of mitochondria is also one of hydrolysis, where it is believed that the mitochondria which come to be included within the food vacuoles bring about the digestion of the food.

We see that *Opalina* and *Monocystis* feed in the same way by means of endosmosis through the cuticle of the protoplasm. One would therefore expect to observe a similar phenomenon in *Monocystis*. But observations on this organism show that the actual origin of these food-storage products from the surface of the mitochondria cannot be detected. The mitochondrial grains present in the protoplasm of *Monocystis* are very much smaller than those of *Opalina*, and it is quite probable that the latter organism, owing to its large size, is the more favourable object for studying this phenomenon. But it is highly suggestive that the vegetative granules, which we previously observed in the residual protoplasm of the early cyst, arise in the same manner as similar plastid and vegetative products in other animal and plant cells.

Bearing in mind the evidence that mitochondria appear to be the centres of general protoplasmic synthesis within the living cell, it is of interest to observe that in *Monocystis* there occurs a stage in the life-cycle of this organism—namely, the spore phase—wherein synthetic activity and digestive activity do not occur, and moreover, at this period previous observations have shown that mitochondria can no longer be detected within the protoplasm of the cell. The study of the behaviour of mitochondria throughout the life-history has shown that these bodies make their reappearance in the free active sporozoite at a time when chemical synthesis takes place. Later, the growth of the sporozoite into the adult individual is accompanied by an increase in the number of cell mitochondria. This evidence seems to suggest that the reawakening of cellular activity within the spore is dependent upon the reformation of mitochondria.

SUMMARY.

1. Observations show that mitochondria are capable of arising *de novo* in the freshly liberated sporozoite stage of the life-cycle of *Monocystis*.

2. Mitochondria are present in large numbers throughout the course of the asexual phase of the life-cycle. During the conjugation of the gametocytes the rod-like mitochondria give rise to numerous spherical bodies. At fertilization the mitochondria within the gametes appear to fuse, resulting in the formation of larger clumps.

At the commencement of the spore phase the mitochondria gradually decrease in numbers, and are totally absent within the mature spore. Later, the growth of the liberated sporozoite or young trophozoite is accompanied by a reformation and rapid reproduction of mitochondria.

3. The disappearance and reformation of mitochondria during certain stages of the life-cycle may be correlated with their apparent synthetic activity.

I am greatly indebted to Professor W. E. Agar, F.R.S., for his valuable suggestions.

This investigation was carried out during the tenure of a Government Research Scholarship in Zoology at the University of Melbourne.

REFERENCES.

1. Cowdry, E. V.—'The American Nat.', vol. lx (1926), p. 135.
2. ——— 'General Cytology.' Chicago, 1924.
3. Guillermond.—'Ann. Sci. Nat., Bot.', vol. x (1919) a.
4. Horning, E. S.—'Australian Journ. of Exper. Biol. and Med. Science', vol. ii (1925), p. 135.
5. ——— Ibid., p. 167.
6. ——— Ibid., vol. iii (1926), p. 89.
7. ——— Ibid., pp. 89, 149.
8. ——— Ibid., vol. iv (1927), p. 69.
9. ——— Ibid., p. 75.
10. ——— Ibid., p. 187.
11. Marston, H. R.—'Biochem. Journ. of Exper. Biol. and Med. Science', vol. 17 (1923), p. 851.

12. Regaud, C., and Mawas.—'Comptes rend. Soc. Biol.', vol. 66 (1926).
13. Scott, W. J. M.—'American Journ. of Anat.', vol. 20 (1916), p. 37.
14. Wallin, I. E.—*Ibid.*, vol. 32 (1924), p. 467.

DESCRIPTION OF PLATE 9.

Sections of *Monocystis* at various stages of the life-cycle. All figures are drawn from material fixed in either Flemming's solution without acetic or else in a Champy fixative and stained with Heidenhain's iron haematoxylin, and occasionally counter-stained in eosin.

Fig. 1.—Longitudinal section of adult individual or Trophozoite, showing mitochondria scattered throughout the medullary region of the organism. Note mitochondria lying close to and in intimate contact with outer surface of nucleus.

Fig. 2.—Section showing association of two adult organisms within the cyst. Note the increase in the number of spherical mitochondria, which have arisen by fusion of the rod-shaped mitochondria of the previous stage.

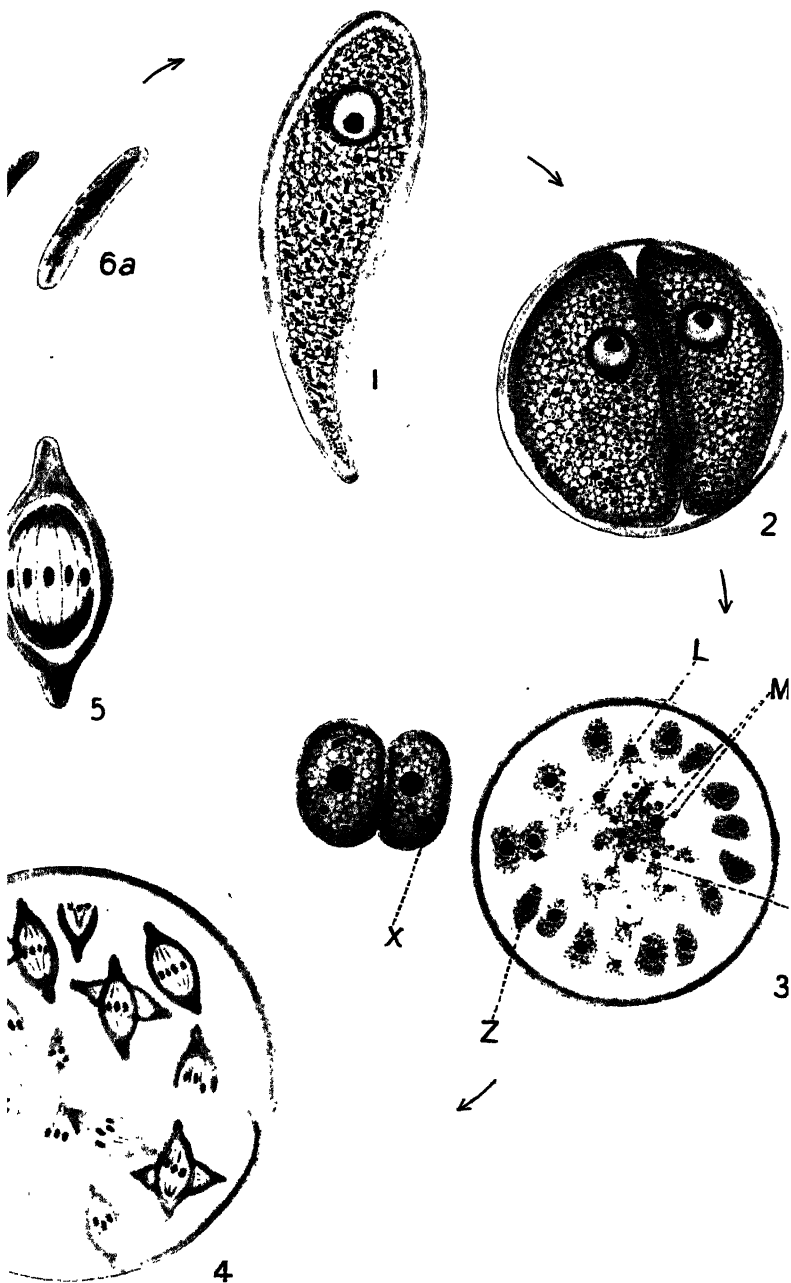
Fig. 3.—Section through cyst showing mitochondria within the protoplasm of the gametes, showing conjugating gametes and larger granular clumps (x and z) formed by fusion of the mitochondria. Within the residual protoplasm are seen the three types of granules. M, mitochondria; L, lipoidal droplets; v, vegetative grains.

Fig. 4.—Section showing mature cyst. The residual protoplasm has been completely absorbed. Note the absence of mitochondria within the spores. The nuclei of spores are stained clearly.

Fig. 5.—Longitudinal section through two spores as seen under higher magnification. Note how the nuclei of each spore stains clearly, and also the total absence of mitochondria.

Fig. 6.—Section cut through a sporozoite showing the reappearance of mitochondria.

Fig. 6 A.—Section of same as seen with higher magnification. Note the rod- and spherical-shaped mitochondria in cytoplasm, together with sharply stained nucleus.



The Conus Arteriosus in Fishes.

By

C. W. Parsons, B.A.

Department of Zoology, University of Glasgow.

With 10 Text-figures.

INTRODUCTION.

THE structure intervening between the ventricle of the heart in fishes and its prolongation beyond the borders of the pericardiac cavity has been described under a number of different names. In literature of the early nineteenth century it occurs as the 'branchial artery' (cf. Home, 10). Other terms such as *bulbus arteriosus*, *bulbus aortae*, and *bulbus cordis* were also commonly applied to it, however, and these have passed into general use.

In a note published in 1842 (15) and again two years later in his important paper on the morphology and definition of the *Ganoidei* (16, p. 127), Joh. Müller emphasized the presence of a muscular covering over the *bulbus arteriosus* in the so-called ganoid fishes and in certain of the *Selachii*. He was followed, 1866, by Gegenbaur (6), who was so deeply impressed with the apparent distinctions, both structural and functional, between the *bulbus arteriosus* in *Selachii* and *Teleostei* that he introduced the special term for the former, '*conus arteriosus*'. ('Ich bezeichne diesen Theil als *Conus arteriosus*, da er dem gleichnamigen Abschnitte der Kammer der höheren Wirbelthiere entspricht.')

Gegenbaur's nomenclature was adopted. Without question his opinion was supported in the strongest possible manner by the evidence he brought together from the comparative anatomy of the heart in adult fishes. When however the more recent facts of the early development of this organ are considered, it

becomes questionable whether the distinction is in reality a true morphological one. The heart is now regarded as a specially modified part of the primitive subintestinal blood-vessel, whose contractile tissues are localized by the boundaries of a comparatively small coelomic space—the pericardiac cavity. The headward or anterior boundary of this cavity is fixed early in development, and with the completion of the posterior wall the developing cardiac tube becomes entirely enclosed. There are some exceptions to this. In certain Selachii and Chondrostei, for example, the pericardiac cavity is never completely shut off from the general body-cavity, and in Myxinoidea the two spaces remain in communication throughout life by a wide lateral opening. As a rule, however, the forerunner of the heart finishes its development within a completely sealed cavity.

It is a commonplace that the growth in length of the heart rudiment and that of the pericardiac space do not keep pace with one another. The heart rudiment grows much the faster and, being fixed at each end by the pericardiac wall, it is thrown into folds. The amount of folding is obviously controlled at any one time by the size of the pericardiac cavity, and it is not therefore surprising to find that the different orders of fishes show much variation in this respect. It is probably most exaggerated in the development of the heart in Dipnoi. Robertson (20, Text-fig. 8, pp. 82, 86), for instance, showed how in *Lepidosiren* the heart rudiment suffers a severe twist in development which is not corrected until later enlargement of the pericardiac space antero-posteriorly releases some of the tension at the extremities of the heart tube.

A fundamental relationship between the anterior and posterior boundaries of the pericardiac cavity and the developing heart is clearly established. Indeed, in a number of the lower Gnathostomata they form indisputably the limits of the adult heart also. There is strong support therefore for the opinion that every part of the cardiac tube within the boundaries of the pericardiac space is to be regarded as morphologically a part of the heart, and it is the point of view adopted here. In conflict with this view is the hypothesis that the *bulbus arteriosus* in

Teleostei is a part of the ventral aorta which has secondarily extended backwards so as to replace part of the cardiac tube. This attractive theory, illustrated by Boas's well-known figure (1, fig. 352, p. 418), receives very general acceptance. But the facts that may be brought forward to show that the bulbus arteriosus in Teleostei is simply part of the original conus arteriosus, i. e. the pre-ventricular portion of the primitive cardiac tube, indicate that the theory ought to be abandoned, and that the explanation of the contrast between the structures evolved should be sought in the substitution of an automatically contractile apparatus in this part of the heart of higher fishes in place of one which had originally independent powers of contraction.

The term conus arteriosus is accordingly used in the following pages in the widest sense to include the whole of the 'morphologically anterior portion of the primitive cardiac tube' (cf. 11, p. 38).

Elasmobranchii.

The conus arteriosus in Elasmobranchii is a well formed straight and important chamber of the heart. It has fairly thick walls and a wide lumen. The presence of a muscular covering—the so-called myocardiac coat—over it has already been indicated (Müller 15 and 16), and correlated with this is the well-known fact that the conus 'contracts rhythmically like the rest of the heart' (cf. Clark 4, p. 23). The myocardiac fibres continue apparently without interruption into the ventricle, where they mingle with similar fibres belonging to the ventricle itself.

The lumen of the conus is occupied to a greater or less extent, according to the species, by a notable development of the endocardiac lining. The tissue expands into loose, almost gelatinous sheets (cf. Gegenbaur 7, p. 352), which are thrown into ridges extending from end to end of the conus. Gegenbaur's figure of a longitudinal section of the conus in a 5 cm. embryo *Acanthias* (8, fig. 6, p. 605), for example, shows two of the wide continuous endocardiac ridges. A similar longitudinal section through the conus of a young specimen 22 cm. in length

shows the ridges segmented into valves. It will be noted that the valves in each longitudinal row are not equally well developed. It will also be clear from the transverse sections 1a and 1b,

TEXT-FIGS. 1, 1a, 1b.

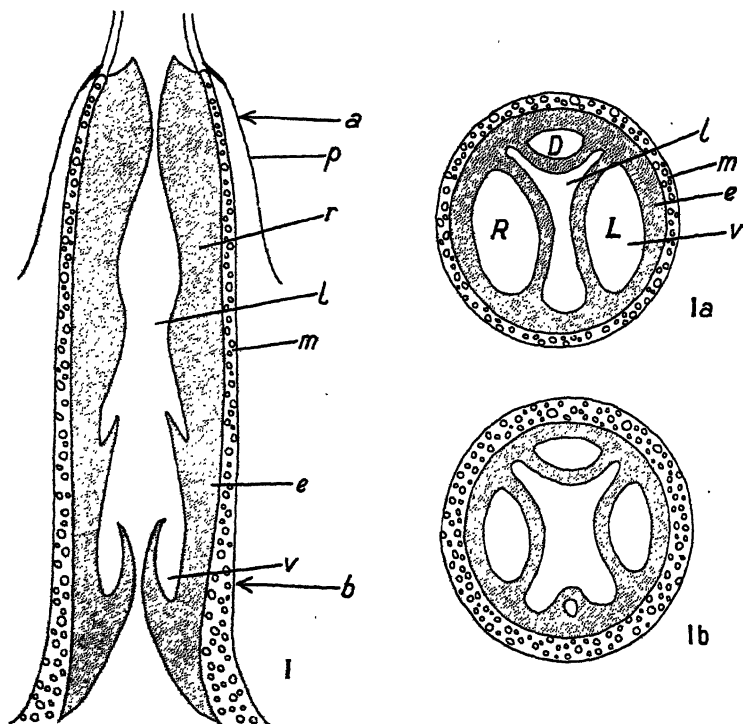


Fig. 1.—Horizontal section of the conus arteriosus of a young *Acanthias*, 22 cm. in length. *a*., region of the transverse section, Text-fig. 1a; *b*., region of the transverse section, Text-fig. 1b; *e*., endocardiac lining of the conus; *l*., lumen of the conus; *m*., myocardiac coat in the conus wall; *p*., pericardiac wall; *r*., ridge of endocardiac tissue between valve circles 1 and 2; *v*., pocket valve.

Figs. 1a, 1b.—Transverse sections of the above in the regions *a*. and *b*.—*D*., *R*., *L*., morphologically dorsal, right, and left valves respectively.

that the number of valves in a circle at the base of the conus is four, and that at the headward end, i.e. the point fixed in

position by the anterior wall of the pericardiac cavity, it is reduced to three.

The convention followed in describing the endocardiac ridges of the conus arteriosus and their derivatives, the valves, may be explained with advantage here. It is a method due to Graham Kerr (12, p. 378) of considering the morphological position of these structures at the headward end of the conus arteriosus and of naming them accordingly. Thus four valves occupying these positions at the fixed headward end are named Right, Dorsal, Left, and Ventral respectively. Abbreviation to R., D., L., and V. is clearly convenient for this particular circle of valves.

Applying this convention to the disposition of the chief conus valves in *Acanthias* (*Squalus*):

1. The valves are in four circles.
2. The right, dorsal, and left endocardiac ridges are present throughout the adult conus arteriosus; the ventral ridge is reduced and appears only in the hinder section of the conus, i. e. nearest to the ventricle.
3. The circles of valves:

Circle 1.—R., D., and L. well-developed pocket valves.

Circle 2.—Reduced valves, or plain ridges, occur in the right, dorsal, and left axes only.

Circles 3 and 4.—Pocket valves in the right, dorsal, and left axes; a smaller pocket valve, or solid strip of endocardiac tissue appears in the ventral axis.

Gegenbaur (6, p. 366) showed that the only valves consistently well developed in the conus of adult *Acanthias* were situated in the foremost and hindmost circles, i. e. the first and fourth. Stöhr (26, p. 212) recognized among the intervening structures two types of valves, namely (1) typical pocket valves, and (2) a form of valve without cavity to which he applied the term tongue valve. No doubt the latter are represented by the strips of endocardiac tissue mentioned above. It is interesting to note that Stöhr found he could distinguish four different grades of endocardiac valve among the *Selachii* and *Ganoidei*, but the intermediate types which he particularized do not

appear to be constant either in position or form, and the distinctions between them are not therefore specially stressed in this paper.

O'Donoghue and Abbott (18, p. 828) describe 'very tiny accessory flaps' in the conus of *Acanthias*. These supernumerary valves are not always present, but when found they will be seen in circles 3 and 4 on one side or other of the dorsal row of valves. These authors do not find units in the ventral axis of the conus and therefore describe the position of the predominant rows of valves as one dorsal and two ventro-lateral rows. A conspicuous feature of the conus arteriosus in *Acanthias* is the wide space between circles 1 and 2 (cf. Gegenbaur, loc. cit., 6, p. 367). It is bridged by thickened endocardiac ridges (cf. Text-fig. 1, r.). An important detail to notice also is the presence of the myocardiac coat over the whole of the conus from its base to the pericardiac boundary.

The conus of the heart in the commoner species of *Raia* resembles that of *Acanthias* in having a fully developed myocardiac covering. There is a difference in the arrangement of the endocardiac valves. In *Raia* there is no wide space between the first and second circles of valves. The number of circles of valves may vary within the genus between four and five more-over (cf. Joh. Müller, loc. cit., 15, and Pettigrew, 19). The latter observed in *R. batis* 'three pyramidal rows of five each', i. e. five circles of valves in three longitudinal rows (cf. 19, p. 779). With this computation Stöhr disagreed. He found only four circles of valves in both *R. batis* and *R. clavata* (loc. cit., 26, p. 219). Stöhr drew attention to a distinction between these two however, in the size of the valves of the first circle as compared with those that follow in the second. In *R. clavata* the valves of the foremost circle are not conspicuous, whereas in *R. batis* they are distinctly larger than those that occur in any of the other circles. Transverse sections through the conus arteriosus of *R. batis* at its apex and base showed no trace of the ventral endocardiac ridge in either situation, and the condition of the endocardiac apparatus in the two species may therefore be summarized as follows :

Raia batis.—Valves in five circles. Pocket valves well developed in every circle.

Circle 1, R., D., L., larger than the remaining valves.

Circles 2–5, three valves in each circle lying on three endocardiac ridges in the right, dorsal, and left axes.

Raia clavata.—Valves in four circles. Pocket valves in each circle about equal in size and their positions identical with those of the corresponding valves in *R. batis*.

In *Scyllium canicula* the muscular covering of the conus in the adult heart does not as a rule extend as far as the pericardiac boundary. It falls short of this fixed point by an amount that appears to vary in different specimens. At its maximum about one-third of the conus may be without its myocardiac coat and the place of it is taken by tissue of the same nature as that which comprises the wall of the ventral aorta beyond the pericardiac boundary. Though comparatively short, this section of the conus has only unstriped muscle in its wall therefore, and if Gegenbaur's distinction between conus and bulbus arteriosus were to be applied strictly, it would be necessary to recognize that both are represented in the conus of this Elasmobranch. The distinction is not only an external one. The development of the endocardiac tissue, and therefore of the valves within the lumen of the conus, closely corresponds with the amount of reduction in the outer myocardiac covering. Thus, three circles of complete pocket valves may be found in the conus of *Scyllium* when the myocardiac coat is at its maximum length (cf. Text-fig. 2b). In cases where this muscular layer is much reduced, however, only two circles of conus valves may be separately identified. Probably it was for this reason that Meckel found only two circles of valves in *Scyllium canicula*, and Stöhr described three (loc. cit., 26, p. 214). A series of transverse sections of the conus do not show more than three complete valves in each circle. Those on the right and left sides of the conus are larger than the valves in the dorsal position. The former tend, especially at the base of the conus, to enclose narrow areas close to the attachments which they make with the conus wall. Outward appearances

then suggest the presence of incipient pocket valves. This however is not the case, for the cavity of any one of the apparently new valves can be shown to be continuous with the cavity of the valve from which it has been derived.

TEXT-FIGS. 2 a, 2 b, 2 c.

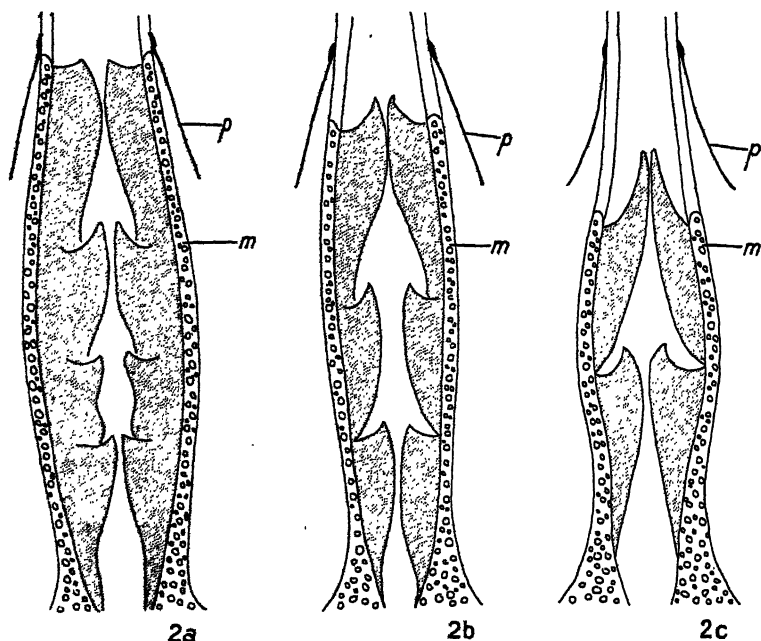


Fig. 2 a.—Horizontal section of the conus arteriosus of *Raia clavata*.

Fig. 2 b.—Horizontal section of the conus arteriosus of *Scyllium canicula*.

Fig. 2 c.—Horizontal section of the conus arteriosus of *Pristiurus melanostomus*.

Showing the reduction in extent of the myocardiac coat *m*. Other structures as in Text-fig. 1.

The position in *Scyllium* is therefore:

Valves in two to three circles. Pocket valves clearly differentiated in every circle. They occur only in the right, dorsal, and left axes.

This formula is true also of the heart of *Pristiurus melano-*

stomus except that the number of circles of valves is not greater than two. In *Pristiurus* moreover, the myocardiac coat in the conus wall is invariably short. As a rule it does not extend over more than half the distance between the base of the conus and the pericardiac boundary. The result is that the base of the first circle of valves is half-way down the conus, and there is a considerable region in front, entirely free from valves, which presumably cannot be rhythmically contractile (cf. Text-fig. 2c).

The four *Elasmobranchii* that have been under discussion thus far clearly show a tendency to reduce the muscular covering of the conus arteriosus. It is notable that hand in hand with this process has gone the virtual elimination of the ventral endocardiac ridge, a reduction in the number of valves in each of the longitudinal rows remaining—from a maximum of five to a minimum of two—and, the commencement in *Scyllium* of a process which resulted in the enlargement of the valves of the morphologically right- and left-hand sides with atrophy of the traces of the dorsal endocardiac ridge also.

Crossopterygii.

Turning to some of the fishes which occupy a rather isolated zoological position, it will be convenient to consider first the condition of the conus arteriosus in *Polypterus*. The figure shows that the proportions of this chamber in comparison with those of the remainder of the heart are far from mean. The myocardiac coat extends forwards over the conus to the pericardiac boundary and the six endocardiac ridges are split up into a very large number of valves. Boas (2, p. 324) recognized three chief longitudinal rows of valves, each comprising nine units, and three lesser rows in between them, also composed of nine small units, i. e. nine circles of valves arranged in six longitudinal rows each of which is made up of three large and three small units. Gegenbaur, on the other hand (loc. cit., 7, p. 356), stated that the number of valves in the foremost circle—circle 1—may vary between two and five. He noted eight further circles, comprising three valves each, and

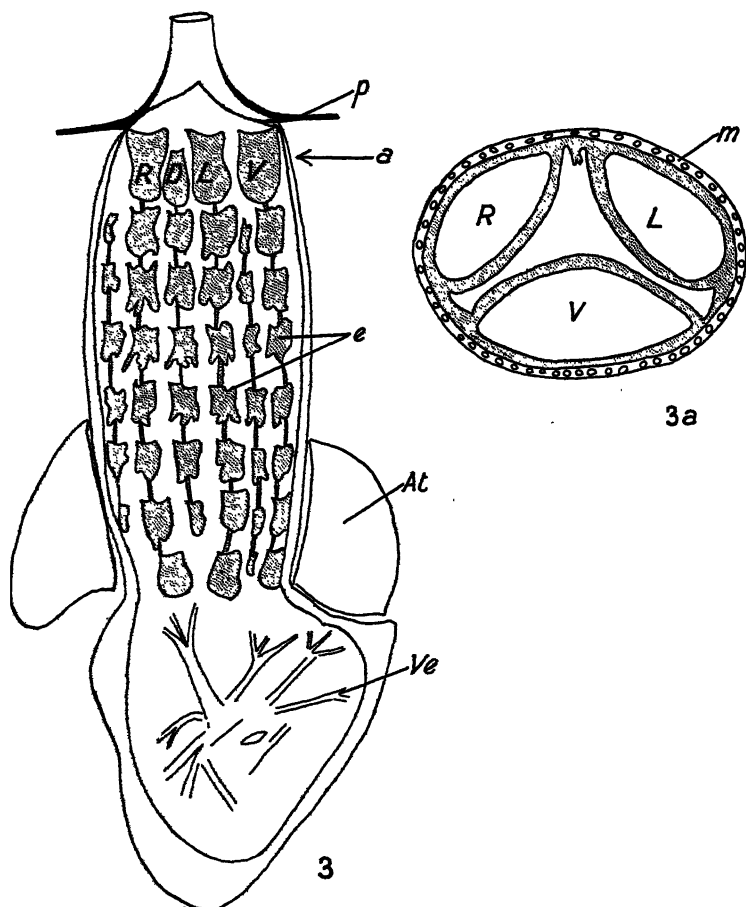


Fig. 3.—Diagram of a ventral dissection of the conus arteriosus and ventricle of *Polypterus*. *R.*, pocket valve in the morphologically right axis. *D.*, pocket valve in the morphologically dorsal axis. *L.*, pocket valve in the morphologically left axis. *V.*, pocket valve in the morphologically ventral axis. *a.*, region of the transverse section, Text-fig. 3 a; *p.*, pericardiac wall; *At.*, atrium; *Ve.*, ventricle; *e.*, endocardiac valves.

Fig. 3 a.—Transverse section of the conus of *Polypterus* in the region *a.* as in Text-fig. 3; *m.*, myocardiac coat.

mentioned that rows of smaller valves occurred in between them. Of the variability in the number of endocardiac valves in this species there can be no doubt—eight circles of valves instead of nine, for example, are shown in Text-fig. 3. There are six longitudinal rows of valves of which only three extend throughout the whole length of the conus. With regard to the circles :

Circle 1 comprises three large pocket valves, R., L., V., and a smaller pocket valve D. The rim of the latter does not reach to the same level as the rims of the other valves in the circle.

Circles 2-7 comprise six valves each, many of which have no cavity. The valves in the central areas of the conus show by their serrated margins a clear tendency to divide further, and the axes they occupy are right, dorsal, left, ventral, and intermediately, between the ventral and right axis and the ventral and left axis.

Circle 8 comprises four pocket valves, three of which lie in the left, right, and ventral axes of the conus and the fourth between the left and ventral axes.

The reduction of the morphologically dorsal valve (D.) at the headward end of the conus in the specimen illustrated and the strong development of the unit in the ventral axis (V.) is brought out by the transverse section, Text-fig. 3a. In this detail is a marked contrast to the arrangement noted in the *Elasmobranchii*, where the ventral axis in the corresponding part of the conus bears no trace of endocardiac tissue.

It is also clear that while in *Polypterus* endocardiac ridges are well developed in each of the four main morphological axes, right, dorsal, left, and ventral, the dorsal ridge and its valve derivatives give signs of decreased usefulness and therefore of approaching elimination.

Holostei.

Very similar to the conus of *Polypterus* is that of *Lepidosteus osseus*. In this species, however, there are typically eight circles of endocardiac valves set in eight longitudinal rows. Four of the latter are developed more strongly than the

TEXT-FIGS. 4, 4a.

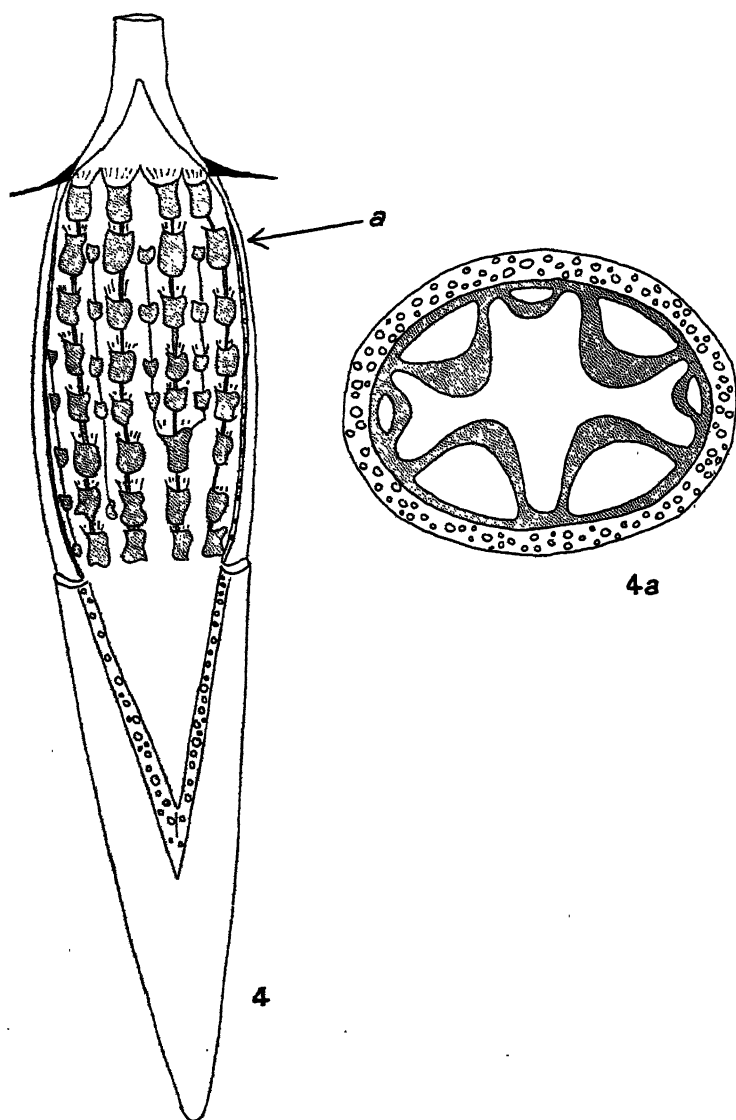


Fig. 4.—Diagram of a ventral dissection of the conus arteriosus and ventricle of *Lepidosteus*. *a.*, region of transverse section, Text-fig. 4a; other structures as in Text-fig. 3.
Fig. 4a.—Transverse section of the above in the region *a.*

remainder and are remarkable because the positions they occupy are intermediate between the right, dorsal, left, and ventral axes.

The arrangement of the circles is shown diagrammatically in Text-fig. 4, thus :

Circle 1 comprises four equally well-developed pocket valves.

Circles 2 and 3 comprise seven valves. Four of these are in line with the intermediate pocket valves of circle 1.

The remainder, in the right, dorsal, and left axes of the conus, are small valves with their cavities poorly developed or quite obliterated.

Circles 4 and 5 resemble the preceding circle with the addition of an extra unit in the ventral axis. The total number of valves in these circles is thus made up to eight.

Circles 6 and 8 comprise five valves. Units are omitted from the right, dorsal, and left axes, but one is present in the ventral axis.

Circle 7 comprises six valves. Five arranged as in the circles 6 and 8, and an extra unit in the right axis.

Variation in the number and position of the minor valves in each circle relative to the dominant valves is to be expected. Stöhr (loc. cit., 26, p. 224) pointed out that they become fewer beyond the fourth circle, and Boas (loc. cit., 2, p. 323) noticed that the small rows of valves do not extend uniformly throughout the conus, &c. Many of the valve units are reduced to solid knots of endocardiac tissue which contrast strongly with the prominent pocket valves of circle 1.

In both *Lepidosteus* and *Polypterus* the individual valves are joined to one another by fibrous connecting strands of tissue. A more strongly marked central ridge of this tissue runs forward from the base of each valve and probably represents the original endocardiac ridge. There are clearly eight of these in *Lepidosteus* and six in *Polypterus*.

Chondrostei.

The clear-cut, well-formed pocket valves in the conus arteriosus of an adult *Acipenser* do not present a very close

resemblance to their homologues in *Polypterus* and *Lepidosteus*. Stöhr (loc. cit., 26, p. 221) described the variation in the number of these valves in different specimens of *Acipenser sturio*. The number of circles of valves (three) was

TEXT-FIGS. 5, 5a, 5b.

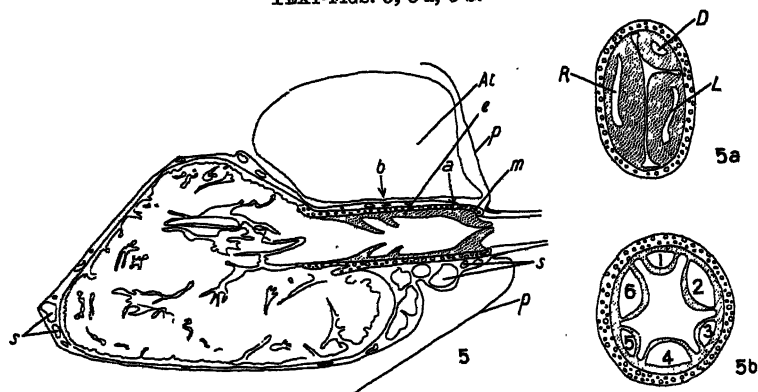


Fig. 5.—Sagittal section of the heart in *Acipenser ruthenus*. *At.*, *p.*, *e.*, as in Text-fig. 3; *m.*, myocardial coat in the conus wall; *s.*, peripheral blood sinuses; *a.* and *b.*, region of transverse sections. Fig. 5a.—Transverse section through the conus arteriosus of *Acipenser* in the region *a.* *D.*, *L.*, *R.*, dorsal, left, and right endocardial valves of circle 1.

Fig. 5b.—Transverse section through the conus arteriosus of *Acipenser* in the region *b.* 1–6, the six pocket valves of circle 2.

identical in all cases; but the number of longitudinal rows was indeterminable owing to the fact that the number of valves in each circle was not constant.

Circle 1 comprised four valves, one of which is said to have been frail or rudimentary.

Circle 2 comprised five to six valves.

Circle 3 comprised four to five valves.

With this formula the arrangement of the corresponding valves in the sterlet *Acipenser ruthenus* and in the 'shovel nosed Sturgeon' *Scaphirynchus platyrhynchus* was found to agree very well. In the former, three valves only occurred in circle 1, lying in the axes *D.*, *R.*, and *L.* (cf. Text-fig. 5a).

There is a long interval between circles 1 and 2 (cf. Text-fig. 5).

Circle 2 comprises six valves (cf. Text-fig. 5 b).

Circle 3 comprises only four valves.

The enlargement of the morphologically right-hand valve (R.) at the headward end of the conus, and the consequent deflexion of the dorsal valve (D.) to the left-hand side, is depicted in the Text-fig. 5 a.

In *Scaphirynchus*:

Circle 1 comprises three well-developed pocket valves and a small solid strip of endocardiac tissue in the morphologically ventral position. The pocket valves, R., D., and L., are so large that they practically occlude the conus lumen by themselves. Partly because of their size and partly because they are set farther from the pericardiac boundary than are the corresponding valves in circle 1 of the sterlet, the interval between these valves and the valves of circle 2 is considerably less than the corresponding interval in the conus arteriosus of the sterlet.

Circle 2 comprises six pocket valves.

Circle 3 comprises four well-developed pocket valves and one solid rudiment.

The external appearance of the heart in both *Acipenser* and *Scaphirynchus* is striking because the wall of the ventricle is enclosed by a loose covering of connective tissue which bears a profuse development of peripheral blood sinuses. This covering spreads over the conus so completely that it gives to it an appearance of being telescoped into the ventricle (cf. Text-fig. 5).

In *Polyodon*, according to Danforth (5), the number of circles of valves may vary between three and four. The number of prominent longitudinal rows, on the other hand, is four.

The myocardiac covering of the conus arteriosus in *Polypterus*, *Lepidosteus*, and *Acipenser* meets the boundary of the pericardiac space. In *Scaphirynchus* it is set back from this fixed point by a small amount, and in *Amia* the process has advanced a great deal farther.

Amia—usually classified with the *Holostei*—stands in an isolated position where the conus arteriosus is concerned. For rather more than half its length the conus wall is built up largely of striped muscle, but the remaining headward portion is composed entirely of smooth, unstriped muscle, without any trace of myocardiac covering. The construction of the whole recalls that of the conus arteriosus in *Pristiurus* although the endocardiac apparatus is not so closely paralleled.

The valves are arranged in *Amia*, as Stöhr (loc. cit., 26, p. 225) and Boas (loc. cit., 2, p. 324) pointed out, in three circles and four longitudinal rows. They are all functional pocket valves but are not all of the same size. The rows of valves in the right and left morphological axes predominate in each circle, and though quite definite, the valves in the dorsal and ventral axes are by comparison very small indeed (cf. Text-fig. 6 a, valves no. 1 and 3).

The biggest pair of all the valves are situated at the headward end of the conus, in circle 1. They are especially notable because a large number of fine fibres, which are fixed to the inner wall of the conus just behind the pericardiac boundary, run backwards to their attachments on the valve margins and traverse the lumen of the conus in doing so (cf. Text-fig. 6, *t.*). The importance of this feature lies in the fact that it may be interpreted as evidence of the former forward extension of the part of the conus that has striped muscle in its wall. The valves in question lie immediately beneath this muscular part of the conus wall, whereas the fibres extend well into the region that has no myocardiac covering. If the view which regards such fibres as due to the destruction of the valve walls is reasonable, their forward attachments are clear indications of the former boundaries of the valves themselves. The association between the foremost circle of endocardiac valves and the headward limit of the myocardiac coat is close in this and other species, and probability seems therefore to favour the view that the endocardiac ridges extended farther forwards in pre-existing forms, and that there went with this, a greater forward extension of the muscular covering.

Amia is not unique in the possession of fibrous connectives between the valves of circle 1 and the conus farther forwards. Boas drew a comparison between *Amia* in this respect and one of the Teleostean fishes (3, p. 529).

TEXT-FIGS. 6, 6 a.

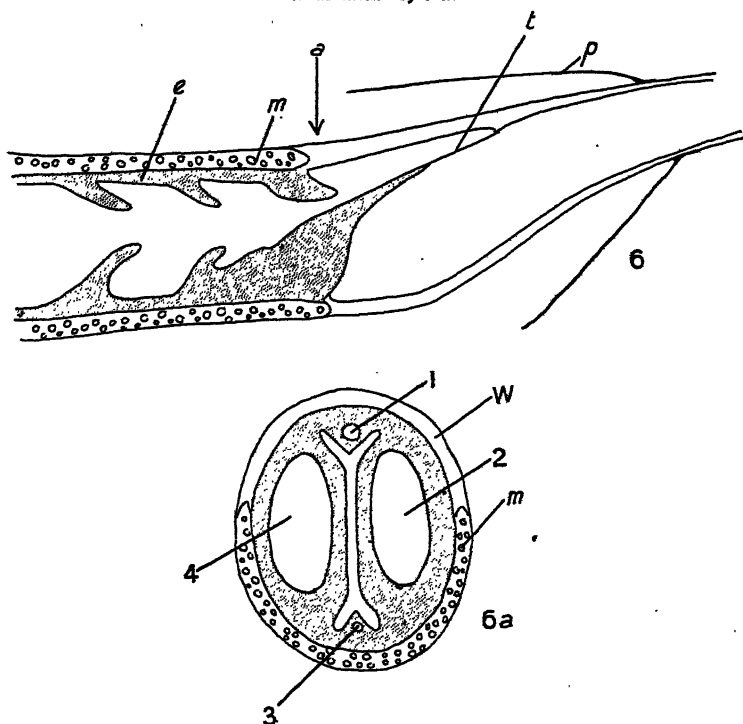


Fig. 6.—Longitudinal section a little towards the right side of the conus arteriosus of *Amia*. *t.*, thread attaching the rim of the right-hand valve in circle 1 to the conus wall. Other lettering as in previous Text-figures.

Fig. 6 a.—Transverse section through the conus arteriosus of *Amia* in the region *a*. Note four pocket valves 1–4, and the junction between the myocardial coat *m.* and the conus wall *W.* of the headward section of the conus.

At this point attention ought also to be drawn to the fact that the development and degree of importance of the pair of pocket valves at the headward end of the conus in *Amia* is paralleled by a corresponding though much less marked develop-

ment of valves in this situation, as compared with the others, in the conus of *Polypterus*, *Lepidosteus*, *Acipenser*, and *Scaphirynchus*.

Dipnoi.

The conus arteriosus in Dipnoi has been the subject of investigation, notably, by Lankester (14, p. 493), Boas (loc. cit., 2), and Robertson (loc. cit., 20). Lankester and Boas were both concerned with *Ceratodus* and *Protopterus*, Robertson with *Lepidosiren*. The whole position has been summarized from the point of view adopted in this paper by Graham Kerr (loc. cit., 11, pp. 40 et seq.; loc. cit., 12, pp. 377-8; 13, p. 388), so that only a few of the more important features of these interesting hearts need comment here.

It is commonly stated in both the older and more recent literature that the conus arteriosus in Dipnoi is twisted. The assertion is made, for example, of the adult heart of *Ceratodus* (cf. Lankester, loc. cit., 14, p. 496, and Nierstrasz, 17, p. 662). The evidence already quoted of the developing heart rudiment in *Lepidosiren* (Robertson, 20, p. 86) shows that this is not so. The characteristic shape of the conus in its final form is to be interpreted rather as a fold or double flexure (cf. 11, p. 41). In *Ceratodus* and *Protopterus* a similar flexure occurs.

With regard to its musculature, the conus in Dipnoi has the third nearest to the ventricle covered with a myocardiac coat which is histologically identical with the muscle of the ventricular wall. At the apex of the first flexure, however, the myocardiac coat thins out perceptibly and continues as a very thin sheet of tissue to the anterior pericardiac boundary. Internal to this remnant of the former muscular wall there lies a layer of tissue similar in every respect to that which forms the wall of the ventral aorta.

There is ample evidence for the opinion that endocardiac valves in the Dipnoi have tended to revert to the primitive condition of endocardiac ridges. The process in this direction has advanced least in the conus arteriosus of *Ceratodus*.

If this is laid open (cf. Goodrich, 9, fig. 219, p. 250) a display of valves will be seen at the headward and posterior ends of the conus flexure that recalls the arrangement of valves in the conus of *Lepidosteus*. The valves at the headward end are disposed in two circles of four units each, those at the base of the conus are in more than four circles; but their number is variable. Boas (loc. cit., 2, p. 329), for instance, calculated a circle of

TEXT-FIG. 7.

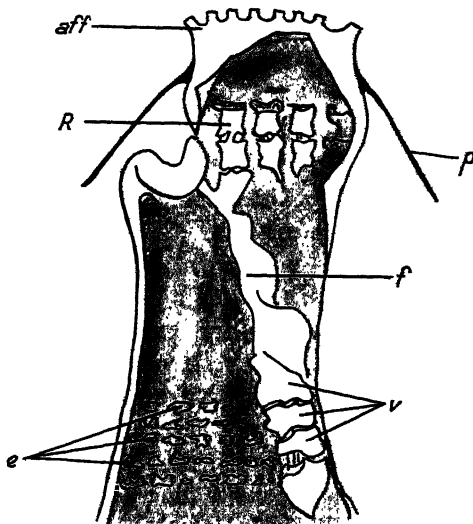


Diagram of a ventral dissection of the conus arteriosus in *Ceratodus*. *aff.*, afferent branchial vessels; *f.*, longitudinal fold—Ridge *R.*; *v.*, valves at the base of the longitudinal fold; *R.*, *p.*, *e.*, lettered as in previous Text-figures.

ten units in one case. By far the most arresting feature of the conus in *Ceratodus* is the great development that has taken place in one of the longitudinal rows of endocardiac valves. It has gone on to the exclusion of all the other endocardiac valves in the central region of the conus. This is of course the region chiefly concerned in the actual flexure. The individual valves in the row in question are distinguishable only at the ventricular end of the conus (cf. Text-fig. 7, *v.*). There are three or

four valves closely bound to one another in this part of the row, and they occupy a ventral position in the adult heart. Beyond the margin of the foremost of these units the row extends as a continuous and strongly developed ridge or fold. The ridge has received various names, e.g. 'spiral valve', 'conus valve', and 'longitudinal fold'. It seems best to adhere to the latter, the name originally employed by Boas. The longitudinal fold, then, reaches across the floor of the conus and round the double flexure. Arriving in the headward section of the conus, it stops short just behind the valve in circle 2 which lies in the morphologically right-hand axis. The conditions in Elasmobranchii clearly show that each longitudinal ridge in the conus was primitively continuous from end to end. It is therefore justifiable to regard the break in the longitudinal fold in *Ceratodus* as secondary, and to name the fold ridge R. for the whole of its extent. The apparent spiral form of this fold is responsible largely for the impression that the conus of the adult heart is twisted. Robertson showed, however, that the similar, though more profound, spiral form of the 'bulbus' valve in *Lepidosiren* was due in development to 'the process of kinking and asymmetrical expansion of an elongated but originally straight bulbus and not to any twisting or counter-twisting of that segment of the heart' (loc. cit., 20, p. 104). It is interesting to note also that the longitudinal fold arises in *Lepidosiren* in two portions discontinuously. One of these belongs to the right wall of the conus, the other to the ventral wall. The former gives rise to the longitudinal fold of the middle and headward portions of the conus, and the latter to the longitudinal fold of the base of the conus, i.e. nearest to the ventricle. There is therefore agreement between *Ceratodus* and *Lepidosiren* on the position of the headward end of this fold although, as has been pointed out, in *Ceratodus* the fold stops short in the headward portion of the conus. Reasons for regarding the fold in *Ceratodus* as representing ridge R. throughout its length have been given, and there is therefore every support for the opinion that the same is true of the longitudinal fold in *Lepidosiren*. In both cases the movement of the fold

from the right to the apparent ventral axis must be due to the conus flexure.

In *Protopterus* Lankester discovered minute traces of valves far down at the base of the conus, but he failed to find any similar traces in the headward portion (loc. cit., 14, pp. 501, 502). The longitudinal fold is prolonged as it is in *Lepidosiren*, and it fuses, in the headward end of the conus, with an almost equally well-developed sheet of endocardiac tissue that lies on the opposite wall in the morphological axis L. (cf. 9, fig. 218, p. 249). The two sheets of endocardiac tissue form a most effective septum which extends backwards to the beginning of the conus flexure.

In *Lepidosiren* again the longitudinal fold fuses at the pericardiac border with a sheet of endocardiac tissue on the opposite wall of the conus, but the septum is not so strongly developed as it is in *Protopterus*. Three persistent rows of vestigial pocket valves in the basal portion of the conus of *Lepidosiren* were noted by Robertson (loc. cit., 20, p. 57).

Teleostei.

At the time when he proposed the name conus arteriosus for the contractile anterior segment of the heart in *Elasmobranchii*, Gegenbaur commented upon the main points in which it differs from the corresponding part of the heart in *Teleostei* (loc. cit., 6, pp. 373-4). The latter is distinguished by the nature of its walls and by the fact that it does not contract rhythmically. A myocardiac covering is entirely absent from the conus in the vast majority of *Teleostei*, and some differences of opinion have been expressed on the manner in which this came about. There are two explanations. The myocardiac coat has been lost either by recession, i.e. intussusception, into the ventricle, or by overgrowth of the mass of tissue in front of it. Both explanations are said to be valid. Smith (24, p. 70) noted that the situation of the valves in relation to the ventricle in some cases favoured one explanation, in others, it favoured the other.

The conus wall in *Teleostei* is built up characteristically of tough connective tissue, plain muscle-fibres, and elastic fibres

which together form a mass varying greatly in size among the different genera. The lumen of the conus also presents a variable appearance. The common Wrasse, the Tarpon, and *Symbranchus* resemble one another, for example, in the possession of a conus with the inner walls ridged by a number of conspicuous plain muscle-strands or trabeculae, which extend from one end of the conus to the other. At the opposite extreme are forms such as the Cod, whose inner conus wall is remarkably smooth. Gegenbaur expressed the opinion that variation of the Teleostei on this point indicated a trend from the ridged towards the smooth condition. Intermediate types are not lacking. The inner wall of the conus of the Salmon, for example, is divided by flat trabeculae at the headward end, but at its base the lining is perfectly smooth. The contrast between the smooth and ridged types of lumen is unmistakable if the figures, Text-figs. 9a and 9c, are compared.

Another very variable feature of the conus arteriosus in Teleostei is the degree of cohesion between the muscular and elastic fibres that form its wall. In trabeculate forms such as *Symbranchus* (Text-fig. 9c) the conus wall is very compact. In the common Dab, on the other hand, the conus wall is composed of a loose spongework through which are innumerable interstices (cf. transverse sections 8b and 8c).

The external appearance of the teleostean conus is also variable. It may be very short and swollen as it is in the Dab, or long and narrow as it is in the Gurnard and *Symbranchus*. The heart of *Gymnarchus* is remarkable because the surface of the conus is moulded into four or more large swellings similar to those shown in Text-fig. 8, cs. But, in spite of the relative differences in external diameter, the nature of the conus wall is constant, i.e. in forms that have an elongated conus the spongework is merely more compressed and its interstices are fewer and smaller.

The endocardiac tissue in Teleostei has a plain surface on which a single circle of valves are typically developed. These are situated at the ventricular end of the conus and may be sunk deeply into the mass of the ventricle itself. There are

some exceptions. Boas (loc. cit., 3, p. 528) reckoned two circles of valves in *Butirinus* (*Albula*) comprising six units in all.

Circle 1 comprises two valves.

Circle 2 comprises four valves; two large valves situated on the right and left wall respectively at the base of the conus, the other two intermediate in position and small.

TEXT-FIGS. 8, 8 a, 8 B, 8 b, 8 C, 8 c.

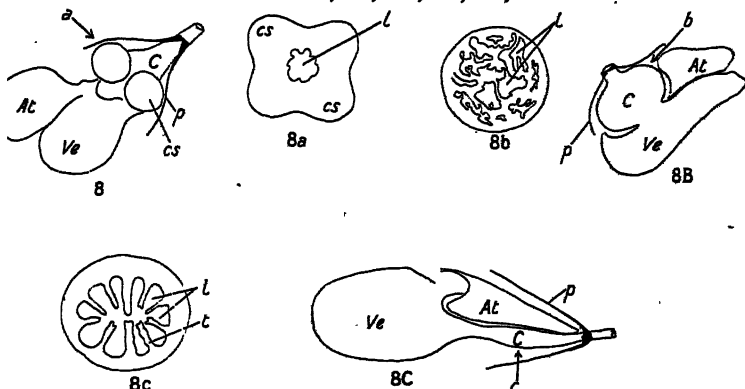


Fig. 8.—Diagram of the heart of *Gymnarchus* excluding the sinus venosus. *At.*, atrium; *Ve.*, ventricle; *c.*, conus; *cs.*, conus swelling; *p.*, pericardiac wall; *a.*, region of transverse section, Text-fig. 8 a.

Fig. 8 a.—Transverse section of the above in the region *a.* *L.*, lumen of the conus; *cs.*, conus swelling.

Fig. 8 B.—Diagram of the heart of the common dab (*Hippoglossoides limandoides*). *b.*, region of transverse section, Text-fig. 8 b; other lettering as in Text-fig. 8.

Fig. 8 b.—Transverse section of the conus arteriosus of the dab. *L.*, lumen of the conus.

Fig. 8 C.—Diagram of the heart of *Symbranchus*. Lettering as in Text-fig. 8.

Fig. 8 c.—Transverse section of the conus arteriosus of *Symbranchus*. *L.*, lumen of the conus; *t.*, trabeculae.

Senior (21, p. 148) described four valves in two circles in the Tarpon, *Megalops atlanticus*, and (22, p. 379) in *Megalops cyprinoides*. The valves are close to the ventricle and are arranged in two longitudinal rows on the right and left sides of the conus (cf. Text-fig. 9 b).

In *Pterothrissus*, again, Senior found two transverse tiers of valves, i.e. valves in two circles (23, p. 84).

The single circle of valves in Teleostei may comprise more than two units. Gegenbaur pointed out, for instance, that while there is only one circle of valves in the conus of the sword-fish *Xiphias*, it is comprised of four units (loc. cit., 6, p. 372).

TEXT-FIGS. 9 a, 9 b, 9 c.

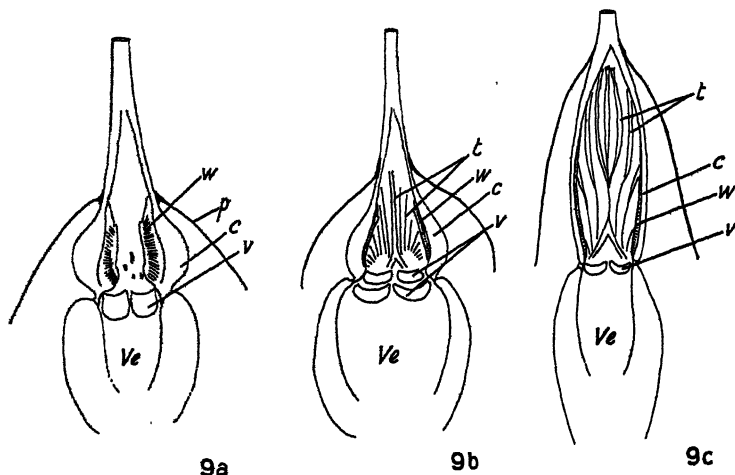


Fig. 9 a.—Diagram of a ventral dissection of the conus arteriosus and ventricle of the common cod (*Gadus morrhua*). *w.*, thick conus wall; *c.*, conus; *p.*, pericardiac wall; *Ve.*, ventricle; *v.*, pocket valve.

Fig. 9 b.—Diagram of a ventral dissection of the conus arteriosus and ventricle of the tarpon (*Megalops atlanticus*). *t.*, trabeculae; other lettering as in Text-fig. 9 a.

Fig. 9 c.—Diagram of a ventral dissection of the conus arteriosus and ventricle of *Symbranchus*. Lettering as in Text-fig. 9 b.

Two of these units are again large valves attached to the lateral conus wall and the other two are small and intermediate in position. Pettigrew, describing a dissection of the heart of a sun-fish, *Orthogoriscus*, mentions three valves in the circle (loc. cit., 19, p. 779), and a similar number is figured (loc. cit., 9, fig. 69, p. 110) in the conus of *Salmo salar*.

Indications of the occurrence of more than two valves in the

conus of Teleostei in the past are not lacking therefore. The existing condition in *Xiphias* makes it at least possible that there were originally four, developed of course from four endocardiac ridges.

Special peculiarities to individual valve systems are not un-

TEXT-FIG. 10.

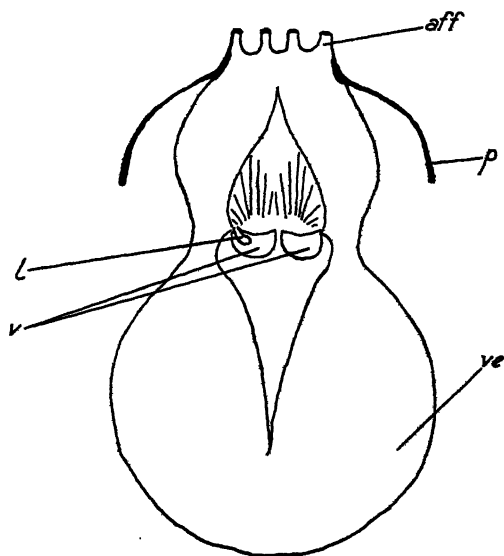


Diagram of a ventral dissection of the conus arteriosus and ventricle of *Lophius piscatorius*. *aff.*, afferent branchial vessels; *p.*, pericardiac wall; *Ve.*, ventricle; *v.*, pocket valve; *L.*, lobe on the right endocardiac valve at the base of the conus.

common, and in this category no doubt there falls a peculiarity of the conus valves of *Lophius piscatorius*. The wall of the right-hand pocket valve bears a conspicuous solid outgrowth which protrudes as a lobe dorsally, and has no doubt the effect of closing off the cavities of the ventricle and conus more perfectly than would otherwise be the case (cf. Text-fig. 10, *L.*).

Cyclostomata.

The conus arteriosus is frequently stated to be absent in Cyclostomata (cf. Nierstrasz, loc. cit., 17, p. 658). It is clearly

represented in the sense in which the term is applied here however, the ventricle of the heart passing anteriorly into a structure homologous with the conus arteriosus of other fishes. A pair of semilunar valves may be found at the base of this structure, and they are developed as in the teleostean fishes on the right and left sides of the cardiac tube in this region.

The walls of the conus are not specially thickened, but they possess, according to Clark (*loc. cit.*, 4, p. 22), considerable automaticity, and they contract on stimulation.

In Myxinoidea the pericardiac cavity remains in wide communication with the main body-cavity, and in Petromyzontidae it also remains open during a large part of the larval existence. The completion of the pericardo-peritoneal septum subsequently seals off the pericardiac cavity in *Petromyzon* however, and it becomes increasingly difficult to delimit the pericardiac boundaries because they are gradually enveloped in cartilage derived from the branchial basket.

The partial development of the pericardiac wall in the early stages of the development of the heart in Cyclostomata must have a considerable effect on the folding of the growing cardiac tube. But the latter remains fixed anteriorly, so that growth in length causes folding and constriction of the tube as it does in other fishes. In comparison the amount of folding is less pronounced, of course, and in the adult, conus, ventricle, and atrium succeed one another in practically a straight line.

In recent work, particularly that of Stensiö (25), the relationship between Myxinoidea and Petromyzontidae is held to be extremely remote. Apart from the difference in respect of the pericardiac cavity which has just been discussed, there is nothing to distinguish between the hearts of present-day representatives of the two groups.

Discussion.

One of the first problems that arises in considering the foregoing description as a whole concerns the number of primitive endocardiac ridges. Only two ridges are present in the Cyclostomata, a group of fishes that are in many respects archaic.

But as this group can claim no close relationship to the Elasmobranchii, and as the number two is characteristic also of Teleostei where it is clearly secondary, there can be no justification for supposing that two was the primitive number of these ridges.

The largest number of endocardiac ridges that have been detailed appear in *Lepidosteus*, where there are eight. In *Polypterus* there are six, and it is suggestive that when the functionally important valves at the headward end of the conus are considered, they should belong in the two cases to eight different morphological axes. In *Polypterus* they are in the positions R., D., L., and V., but the representative of the dorsal axis is considerably reduced. In *Lepidosteus* the chief valves of circle 1 occupy the four axes intermediate between R. and D., D. and L., L. and V., and V. and R. The morphologically dorsal ridge is very strongly developed in Elasmobranchii, and it must be counted in trying to estimate the largest number of axes of functional significance. The possibility that the primitive number of ridges may have been at least as high as eight cannot therefore be passed over. Gegenbaur (loc. cit., 8, p. 610) was inclined to admit it in spite of his own evidence of only four endocardiac ridges in an embryo *Acanthias* (loc. cit., 8, p. 605). Confirmatory evidence from the embryos of the fishes cited is still wanting, however, and without it nothing more positive can be said in favour of the view that the primitive number of endocardiac ridges in the conus may have been so high.

On the other hand, O'Donoghue and Abbot (18, p. 829) have shown that four longitudinal rows of valves are common in the adult heart of the least specialized Elasmobranchii—including Notidanidae. The evidence from Gegenbaur's embryo *Acanthias* may be interpreted as support for the view that the primitive number of ridges was four, and there seems no doubt that 'four was the number present in primitive Tetrapoda' (loc. cit., 12, p. 388). Strong reasons exist, therefore, for supposing that the primitive number of these ridges in fishes was four. This is the number indicated by the presence of four longitu-

dinal rows of valves in such various forms as *Polyodon*, *Amia*, and *Xiphias*, and if the view is correct, the larger number of ridges characteristic of the conus in *Lepidosteus* and *Polypterus* must have arisen secondarily.

The evolutionary importance of conus valves has been the subject of much discussion. They differ much in different types and are valuable links in phylogeny.

Starting with *Elasmobranchii* as the most primitive group, it is clear that the evolution of the conus has followed two different paths.

1. Those forms which have a fully developed muscular conus wall point to a tendency to multiply the number of conus valves whilst retaining full powers of contractility. In contrast to this,

2. Other *Elasmobranchii*—such as *Pristiurus*—show the existence of the opposite tendency, namely, to localize the contractility of the conus and at the same time to reduce the valve apparatus it contains.

The first route obviously leads to greater complexity and the second to greater simplicity of structure. The additional valves that have been shown to occur in either of the circles nearest the ventricle in *Acipenser* make of this conus an interesting link between the condition described in *Polypterus* with six longitudinal rows of valves and *Lepidosteus* with eight. The myocardiac coat in all three genera extends forwards over the conus as far as the pericardiac boundary, and in this characteristic the conus in *Dipnoi* is similar. The dipnoan condition probably evolved from a complex form of conus like that of *Lepidosteus*. It has been shown that the characteristic longitudinal fold of the dipnoan conus is a composite structure, and the traces of more than four valves at the base of the conus in *Ceratodus* may be taken as evidence pointing to ancestry of a kind that was complex in the sense of having many endocardiac ridges.

The sequence may be advantageously expressed thus :

Lepidosteus
Acipenser—— or ——Dipnoi
Polypterus

and thence to terrestrial vertebrates.¹ At each step the conus arteriosus becomes fundamentally more complex.

The commencement of variation in the opposite direction is indicated in Elasmobranchii by Scyllium and Pristiurus. In *Amia* the outward sign of this tendency, i. e. restriction of the myocardiac covering, is particularly marked. Gegenbaur, Boas, and others, recognized that upon the available evidence the only possible position for *Amia*, as far as the conus is concerned, lies between Elasmobranchii and Teleostei. If this is its true place, the single pair of valves in the conus of Teleostei must be equivalent to the well-developed pair of them at the headward end of the conus in *Amia*. The fibrous connexions of the latter with the wall of the conus in front of them has already been emphasized as evidence for the assertion that the contractile part of the conus formerly extended farther forwards, and this assertion must be equally true of the conus in Teleostei. The sequence Elasmobranch—*Amia*—Teleost is less abrupt than appears at first because of the few exceptional teleostean fishes that have more than a single pair of conus valves. It is also a frequent experience to find the conus of a teleostean fish no more muscular apparently than that of *Amia*.

In view of the very great differences in the muscular development of the conus arteriosus in Teleostei, the question of its function becomes a matter of interest. Ingenious suggestions were made on the matter long ago, by Home (loc. cit., 10, p. 235). He related the muscularity of the conus in a particular fish with the depth of water in which it habitually swims. The first consideration, however, must be the maintenance of an efficient circulation through the finer blood-vessels of the gills. Obviously the blood should course through these fine vessels with an even pressure, and it seems probable that the advantage gained by the Teleostei in substituting an elastic apparatus for the contractile type of conus has to do with the attainment of

¹ The generic names are used to indicate phases in cardiac evolution, i. e. there is no suggestion that genera such as *Lepidosteus* or *Polypterus* are descended from *Acipenser*.

a measure of independence of the heart beat. The function of the so-called carotid gland in *Amphibia* has a similar significance. It acts apparently as a buffer to the blood-pressure in the artery on the heart side and maintains a steady flow of blood to the head.

The problem of the occurrence of a single pair of conus valves in *Cyclostomata* has yet to be approached embryologically. If the thesis here maintained of a primitive arrangement of four endocardiac ridges is sound, it must be presumed that a parallel evolution to that of the conus in *Teleostei* is responsible for the reduction of this number to two in both cases.

SUMMARY.

1. The term 'conus arteriosus' is used to define the whole of the headward portion of the heart in fishes which intervenes between the ventricle and the anterior boundary of the pericardiac space.

2. A brief description of the conus arteriosus in a number of different forms is given and attention is directed particularly (a) to the musculature of the conus wall, (b) to the number of endocardiac ridges or valves which it contains, and (c) to the position of these ridges in relation to the headward boundary of the pericardiac cavity. The latter is taken as a fixed point morphologically and therefore as giving true indications of the positions of the ridges (right, dorsal, left, and ventral). Thus, in *Dipnoi*, the conspicuous longitudinal ridge or fold which forms the conus septum is morphologically right in position. The hearts examined include those of *Acanthias*, *Raia*, *Scyllium*, and *Pristiurus*, *Polypterus* and *Lepidosteus*, *Amia*, the *Dipnoi*, and various *Teleostei*. Among the latter are *Megalops*, *Gymnarchus*, and *Symbranchus*.

3. The tendency to reduce the contractile function of the conus and to restrict its valves to the ventricular end is noted even within the *Elasmobranchii*. The process has advanced so far in *Teleostei* that the entrance to the lumen of the conus is

protected by only one pair of valves as a rule, and the conus has lost its myocardiac covering.

Megalops is one of a small number of exceptional Teleostei in which the endocardiac valves are more numerous.

4. The number and position of endocardiac valves in Cyclostomata is similar to their number and position in Teleostei.

5. Discussion is confined (1) to the primitive number of endocardiac ridges; (2) to the evolutionary significance of the conus arteriosus in fishes; (3) to the function of the conus arteriosus in Teleostei.

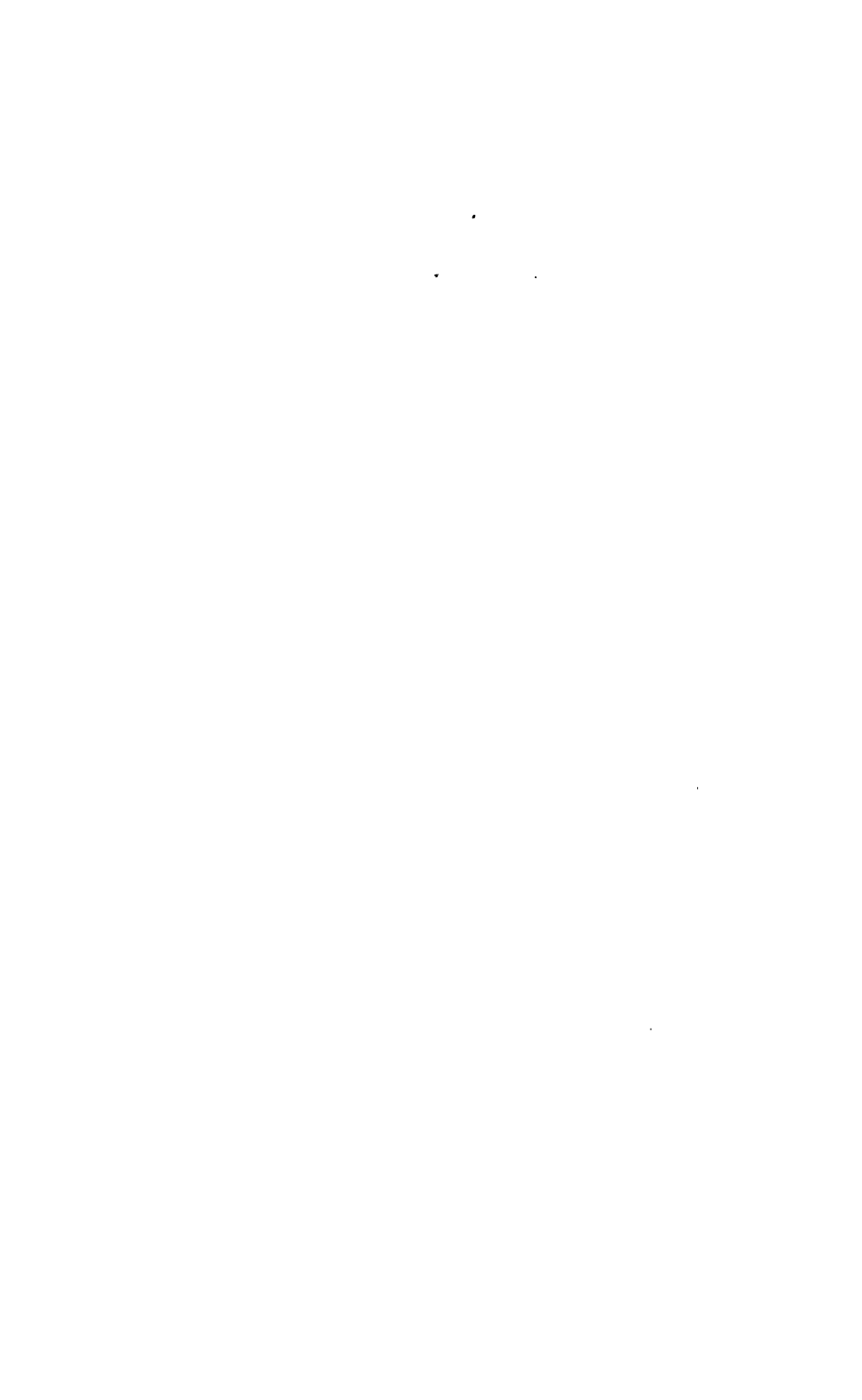
ACKNOWLEDGEMENTS.

I desire to acknowledge my great indebtedness to Professor Graham Kerr for his advice in directing this work, his kindness in reading the manuscript, and constant encouragement. I am also very grateful for material received from the Superintendent of the Scottish Marine Biological Association, Mr. Michael Graham, and Dr. G. S. Carter.

LITERATURE.

1. Boas, J. E. V.—'Lehrbuch der Zoologie.' Jena, 1901.
2. ——— "Über Herz und Arterienbogen bei *Ceratodus* und *Protopterus*", 'Morph. Jahrb.', vol. vi, 1880.
3. ——— "Über den Conus arteriosus bei *Butirinus* und bei anderen Knochenfischen", *ibid.*
4. Clark, A. J.—'Comparative Physiology of the Heart.' Cambridge, 1927.
5. Danforth, C. H.—"The Heart and Arteries of *Polyodon*", 'Journ. Morph.', vol. 23, 1912.
6. Gegenbaur, C.—"Zur vergleichenden Anatomie des Herzens.", 'Jena. Z. f. Naturw.', vol. ii, 1866.
7. ——— 'Vergleichende Anatomie der Wirbelthiere', vol. ii. Leipzig, 1901.
8. ——— "Über den Conus arteriosus der Fische", 'Morph. Jahrb.', vol. xvii, 1891.
9. Goodrich, E. S.—"Vertebrata Craniata, 1st fasc., Cyclostomes and Fishes", 'Treatise on Zoology', ed. by Lankester, Part IX. London, 1909.
10. Home, Sir E.—"Additions to an account of the anatomy of *Squalus maximus*", 'Phil. Trans. Roy. Soc.', vol. cii, 1813.

11. Kerr, J. Graham.—“Note on the morphology of the heart of Vertebrates”, ‘Proc. Roy. Phys. Soc. Edinb.’, vol. xix, 1913.
12. ——— ‘Text-book of Embryology,’ vol. 2. London, 1919.
13. ——— ‘Zoology for Medical Students.’ London, 1921.
14. Lankester, Sir E. Ray.—“On the hearts of *Ceratodus*, *Protopterus*, and *Chimaera*”, ‘Trans. Zool. Soc. Lond.’, vol. x, 1879.
15. Müller, J.—“Bemerkungen über eigenthümliche Herzen des Arterien- und Venensystems”, ‘Arch. Anat. Physiol. (Berlin)’, Jahrg. 1842.
16. ——— “Über den Bau und die Grenzen der Ganoiden”, ‘Abh. der k. Akad. Wiss. Berlin’, 1844.
17. Nierstrasz, H. F.—‘Vergleichende Anatomie der Wirbeltiere’, von Ihle, Van Kampen, Nierstrasz, und Versluys. Translated G. Chr. Hirsch, Berlin, 1927, from the Dutch.
18. O’Donoghue, Charles H., and Abbott, Eileen.—“The Blood Vascular System of the Spiny Dogfish”, ‘Trans. Roy. Soc. Edinb.’, vol. lv, 1928.
19. Pettigrew, James Bell.—“On the Relations, Structure, and Function of the Valves of the Vascular System in Vertebrata”, *ibid.*, vol. xxiii, 1864.
20. Robertson, Jane I.—“The Development of the Heart and Vascular System of *Lepidosiren paradoxa*”, ‘Quart. Journ. Micr. Sci.’, vol. 59, 1913.
21. Senior, H. D.—“The Conus Arteriosus in *Tarpon atlanticus*”, ‘Biol. Bull. Woods Holl’, vol. 12, 1906–7.
22. ——— “Note on the Conus Arteriosus of *Megalops cyprinoides*”, *ibid.*
23. ——— “Teleosts with a Conus Arteriosus having more than one row of valves”, ‘Anat. Rec.’, vol. 1, 1907.
24. Smith, Wilbur C.—“On the process of disappearance of the Conus Arteriosus in Teleosts”, *ibid.*, vol. 15, 1918.
25. Stensiö, Erik A.—“The Downtonian and Devonian Vertebrates of Spitsbergen”, ‘Skrifter om Svalbard og Nordishavet’, vol. 12, 1927.
26. Stöhr, Phil.—“Ueber den Klappenapparat im Conus arteriosus der Selachier und Ganoiden”, ‘Morph. Jahrb.’, vol. ii, 1876.





Russell photographer

Ernest Wilkes Ltd. photo

Sincerely yours
E. Ray Lankester

EDWIN RAY LANKESTER

Born on May 15th, 1847

Editor of THE QUARTERLY JOURNAL
OF MICROSCOPICAL SCIENCE
from 1869 to 1920

Honorary Editor since 1921

Died on August 15th, 1929

Nucleolar phenomena during Oogenesis in certain Tenthredinidae.

By

R. A. R. Gresson, B.Sc.

From the Department of Natural History, University College (University
of St. Andrews), Dundee.

With Plate 10 and 1 Text-figure.

CONTENTS.

1. INTRODUCTION	177
2. PREVIOUS WORK	177
3. MATERIAL AND METHODS	182
4. NUCLEOLAR EMISSIONS	182
5. DISCUSSION	187
6. SUMMARY	190
7. CONCLUSIONS	191
8. ACKNOWLEDGEMENTS	192
REFERENCES	192
EXPLANATION OF PLATE	194

1. INTRODUCTION.

THE present work was undertaken for the purpose of determining the nature of the nucleolar buds described in a recent paper on saw-fly oogenesis (Peacock and Gresson, 20). While this paper is confined to the staining reactions and phenomena associated with the liberation of the nucleolar emissions, it is hoped, in some future contribution, to deal in some detail with yolk-formation and the history of the cell-inclusions during Tenthredinid oogenesis.

2. PREVIOUS WORK.

In studying the literature relating to nucleolar phenomena, one finds no complete summary of the more recent results in any one paper, so that it is advisable to give such in a brief statement here.

Ludford (12), working on *Patella*, states that the early oocyte contains an oxyphil nucleolus. As growth proceeds this increases in size and gives rise to oxyphil emissions which pass into the cytoplasm. These extrusions appear to dissolve, 'as different fragments show various intensities of coloration'. At the end of the preliminary growth stage the nucleolus becomes separated into two parts, one oxyphil, the other basophil; during this process there is an extrusion of oxyphil substance into the cytoplasm. After the differentiation of the basophil nucleolus, the two parts may break up, they may remain joined, or they may separate and form distinct oxyphil and basophil nucleoli. When the yolk becomes fairly evenly distributed the two parts of the nucleolus become vacuolated and begin to disintegrate, the oxyphil part fragments and passes out into the cytoplasm leaving only a small part in the nucleus, while the basophil part disintegrates and becomes spread over the linen network.

As the early nucleolar extrusion takes place before the dispersion of the Golgi elements, Ludford suggests that the emissions may in some way prepare the cytoplasm for the activities of the Golgi elements during yolk-formation.

Jørgensen's findings (Ludford, 12) for a species of *Patella* differ somewhat from those of Ludford.

The nucleolus of the youngest oocytes is apparently amphophil, two kinds of emissions are given off from this, oxyphil granules and amphophil bodies which develop a basophil cap. These become scattered through the nucleus and eventually arranged peripherally as 'Randnukleolen'.

Ludford (13), working on *Limnaea stagnalis*, found the behaviour of the nucleolus closely similar to that of the *Patella* with which he worked.

Gatenby (5), describing the oogenesis of *Saccocirrus*, has shown that the nucleolus gives rise to buds which pass through, and become attached to the outside of the nuclear membrane; these eventually lose their connexion and passing into the cytoplasm become broken up into granules which give rise to yolk or 'nucleolar deutoplasm'.

Closely similar phenomena have been observed in many different groups; thus King records the occurrence of nucleolar buds in *Peripatopsis capensis* (10) and in *Lithobius forficatus* (9). In the former the buds have not been observed to pass through the nuclear membrane, while in the latter the young oocytes give rise to buds which pass out into the cytoplasm and 'disappear shortly after leaving the nucleus'. Later, 'the nucleolus breaks up into a number of pieces', these 'enlarge and start a process of budding', the nucleolus becoming fragmented into a number of small grains. These grains increase by budding in the cytoplasm, and eventually enlarge and form yolk-spheres. Nath (16) states that in *Lithobius forficatus* the nucleolus is at first basophil, then 'amphophil and finally acidophil'; the nucleolar extrusions, he believes, contribute towards yolk-formation.

Nath's description for the oogenesis of scorpions (18) is worthy of note; he finds that in *Euscorpium napolii* and *Buthus judacius* the nucleolus gives rise to 'deeply basophil bodies' which pass into the cytoplasm.

In these two species ordinary yolk is present (proteid in nature) but is absent from the oocytes of *Palamnaeus fulvipes madraspatensis*. In the latter species no nucleolar budding takes place, in the former the nucleolar extrusions become acidophil and 'ultimately disappear as whole bodies', their substance probably taking part in yolk-formation.

Nucleolar emissions have been described by several workers on insect oogenesis; thus McGill (14), for dragon-flies, states that in *Plathemis lydia* oxyphil bodies pass out from the basophil nucleolus; these, however, 'dissolve in nuclear sap, and there is ground for concluding that this material undergoes a chemical change and is reprecipitated as the chromatin-reticulum'. In *Anax junius* there is only one oxyphil body which persists throughout the growth period. The basophil nucleolus represents the chromatin of the egg and during growth forms a spireme which later gives off granules which form a dense chromatin network in the nucleus.

Hogben (8), for *Periplaneta*, describes two kinds of bodies

as arising from the nucleolus. The early oocytes contain a single nucleolus which is at first spherical, and as indicated by methods of staining is a plasmosome. From the early growth period this body emits small deeply staining particles, which pass through the nuclear membrane and move towards the periphery of the egg. At a certain stage vacuoles appear in the plasmosome, later they become granular and more chromatic, having a closely similar appearance to small nuclei within the nucleolus. These bodies or deutosomes pass out from the nucleolus, through the nuclear membrane and to the periphery, and at the same time it seems probable that the first type of emission ceases.

The vacuolation of the plasmosome synchronizes with the first appearance of the yolk-spheres, which is preceded by the formation of vacuoles at the periphery of the egg; within some of the latter chromatin granules given off from the nucleolus were observed. Hogben concludes that true secondary nuclei are not present in this species. The deutosomes 'break up into several homogeneous globules, which are the yolk-spheres', the first globules deposited at the periphery are not homogeneous but are closely similar to the intranuclear deutosomes. In certain Hymenoptera a similar process was observed 'although no account was given, in view of the desirability of examining more favourable material with more suitable technique'.

Nath (17) finds that the early oocytes of *Culex* contain an amphinucleus consisting of a central basophil part surrounded by a plastin portion. The latter part becomes ovoid and then branches in an irregular manner; the branches fill up the nucleus and ultimately become detached from the nucleolus, which now consists of the basophil portion only. Vacuoles appear in the basophil part and its staining properties change. 'The amount of nucleic acid in the nucleolus of the oocyte of *Culex fatigans* immediately after emergence from the pupa is so great that it will stain with basic dyes only.' As the growth of the oocyte proceeds 'the nucleic acid seems gradually to leave first the outer portion of the nucleolus, which therefore begins to stain with acid dyes, and ultimately the central portion also'.

Nakahara (15), working on the silk glands of *Pieris rapae* and *Neuronia postica*, states that before the silk glands become functional portions of the nucleoli pass from the nucleus into the cell in considerable numbers, these emissions stain with acid dyes, and, he suggests, give off phosphorus as they pass from the nucleus.

Gardiner (3) describes an interesting condition in *Limulus polyphemus* where the nucleolus is formed by the coalescence of bodies which pass into the nucleus. The central region is acidophile, the periphery basophil. Later, nucleolar emissions take place.

‘There is no indication that these buds are constricted off as such, it seems rather that the condition is one in which the internal pressure of the nucleolus has risen to such a degree that some of its substance is forced out—a process comparable to the bursting of a bubble.’

These spheres pass out into the cytoplasm and to the periphery, where they disappear as wholes without any fragmentation. There is a small acidophil region round each emission just before it disappears; the substance goes into solution in the cytoplasm and imports to it something which alters its staining reaction. As the result of tests on fresh and fixed material, Gardiner concludes that the nucleolus contains stores of ‘some organic substance very rich in phosphorus, and that this compound is given off to the cytoplasm during deutoplasmogenesis’. The compound contributes to the synthesis of the definitive yolk. Nath (19) in a recent paper on spider oögenesis finds that the nucleolus of the youngest oocyte ‘is highly basophil’. It increases in size and ‘develops vacuoles inside it, and becomes acidophil. It may bud off another nucleolus from it. . . . There are no nucleolar extrusions.’

To summarize, oocyte nucleolar extrusions are recorded as occurring in many different groups, and are believed by several workers to take part in yolk-formation. These emissions are oxyphil in *Patella* (12) and some other forms, while in *Euscorpius napolii* and *Buthus judacius* (18) basophil buds only are present.

McGill (14) believes that in *Plathemus lydia* the oxyphil bodies given off from the basophil nucleolus undergo a change and are 'reprecipitated as the Chromatin-reticulum', while in *Anax junius* the basophil nucleolus gives off granules which form a chromatin network in the nucleus.

3. MATERIAL AND METHODS.

Material was obtained from specimens of *Thrinax macula* Kl. and *Allantus (Emphytus) pallipes* Spin. (Enslin, 2); the former in April and May 1928, from pupae and adults which had passed the winter in the pupal condition, the latter from pupae and adults in June and July 1928.

The following procedure was adopted. The ovaries were dissected out under tap-water and fixed immediately in corrosive-acetic fixative. Sections 5μ in thickness were stained in Mann's methyl-blue-eosin. The above fixative and stain were found to be the most satisfactory, the oxyphil nucleolus being stained pink and the basophil nucleolus blue.

Material fixed by Champy-Kull's, Da Fano's, and Bensley's technique, and in Bouin's picro-formol was used for comparison. Two figures are also given of sections from an adult specimen of a sister species, *Thrinax mixta* Kl. This material was fixed in Bouin's picro-formol and stained in iron haematoxylin.

It should be remarked that the material was taken from parthenogenetic females. In *Thrinax macula* males have been reared, but it has been found (21) that at least two kinds of females exist, one male-producing, the other female-producing. In *Allantus pallipes* several generations have been reared parthenogenetically without the appearance of a single male, nor have males been found in nature.

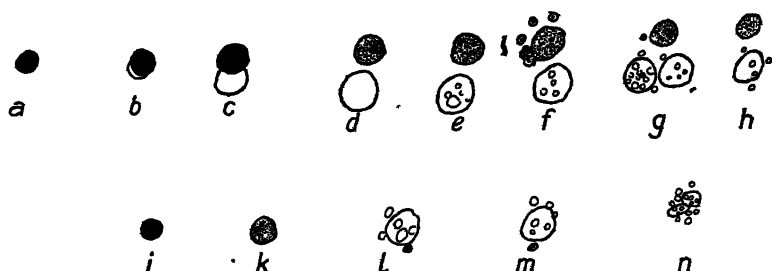
4. NUCLEOLAR EMISSIONS.

The ovarian tubes of a *Thrinax macula* pupa, stained with Mann's methyl-blue-eosin, revealed some interesting facts. Yolk-formation had only just commenced in a few of the older oocytes, but all stages between this and the small recently

differentiated oocytes at the proximal end of the tubes were shown.

In the early oocytes before the formation of the nutritive chambers the nuclei stain pink, while the nucleoli are basophil (fig. 1, Pl. 10). At a later stage the nucleolus has increased in size, and, at the same time, has developed a slightly oxyphil margin around the basophil part present in the older cells (fig. 2, Pl. 10). The oxyphil part increases in size and in staining properties and

TEXT-FIG. 1.



Diagrammatic representation of nucleolar phenomena.

Black and stippled = basophil nucleolus and buds.

Clear outline = oxyphil nucleolus, vacuolated body, and buds.

a-h = *Thrinax macula*; *i-n* = *Allantus pallipes*.

a, original basophil nucleolus of early oocyte; *b*, early stage in differentiation of oxyphil nucleolus; *c*, later stage of same; *d*, oxyphil and basophil nucleolus separate, the latter now consists of a darkly staining granule surrounded by faintly staining material; *e*, showing formation of buds in oxyphil and basophil nucleolus; *f*, basophil buds free in nucleus; oxyphil buds not yet liberated; *g*, showing large vacuolated body which has originated from the oxyphil nucleolus; although a basophil bud is shown the vacuolated body may not be formed until a later stage; *h*, late stage after yolk-formation. The basophil nucleolus is no longer active; the oxyphil nucleolus is still giving rise to buds; *i*, original basophil nucleolus of early oocyte; *k*, later stage during which the nucleolus is faintly basophil; *l*, later stage: the nucleolus is now oxyphil and is giving rise to buds. A basophil body is shown close to the nucleolus; *m*, later stage after yolk-formation; oxyphil nucleolus budding; basophil body without granules present; *n*, showing a condition observed in many of the late oocytes, the oxyphil nucleolus appears to be breaking up.

becomes rounded off from the other part of the nucleolus; this is shown in fig. 3, Pl. 10, taken from an oocyte which is sharply marked off from the nurse-cells. The two parts of the nucleolus

may remain in close association for some time, or, as is more usual, become separate, the basophil part consisting of a small round body or dark granule surrounded by a faintly staining, vacuolated body which appears to vary slightly in its staining reactions, being in some cases faintly blue, while in others the acid stain seems to predominate. The basophil stain appears to become more marked in the older oocytes although, in the greater number of nucleoli examined, the outer margin seemed to be more strongly oxyphil than the remainder. The oxyphil nucleolus or plasmosome increases greatly in size, and is roughly spherical in shape (fig. 4, Pl. 10). The next stage is clearly shown by two oocytes from an ovarian tube of an adult. (It should be noted that the adult tubes, although containing fully developed eggs, also contain oocytes which are directly comparable to corresponding stages found in the pupa.) The vacuoles in the outer part of the basophil nucleolus become more marked and in some cases contain small dark granules similar in staining properties to the round basophil body from which they are apparently given off (figs. 5 and 6, Pl. 10). On examining some of the older oocytes from the pupal material, these granules were seen to increase in size, pass towards the periphery, and finally become liberated as separate bodies consisting of a basophil granule surrounded by slightly basophil material; the latter part, however, may be slightly oxyphil at the time it is liberated (fig. 7, Pl. 10). These buds may have another manner of origin as suggested by fig. 8, Pl. 10; here a large vacuolated mass is shown in contact with the basophil nucleolus from which it appears to have been derived. Granule-containing vacuoles occur in this mass, the larger bearing a striking resemblance to buds occurring free in the nucleus and to those already described as originating from the basonucleolus. Thus it would seem that vacuolated masses may be given off from the nucleolus, and that these in turn give rise to buds containing a basophil granule.

In a last oocyte of the pupal material some buds were shown free in the nucleus and apparently moving towards the nuclear membrane (fig. 9, Pl. 10). In another oocyte they were observed close to the nuclear membrane (fig. 10, Pl. 10), but in no case

were they shown passing through it or situated in the ooplasm. At this stage the latter is deeply basophil and would render the detection of the buds, if present, very difficult. Their position, however, suggests that they may pass into, or give some of their substance to, the ooplasm.

As the dark granules deriving from the basophil nucleolus are spreading through the outer part of the latter, the plasmosome enters upon a period of activity. Thus, several oxyphil bodies appear on its surface and although clearly marked off they may not become detached at this stage (fig. 6, Pl. 10). This process continues during the liberation of the basophil emissions (figs. 7 and 8, Pl. 10), and in some cases, it would appear, a large oxyphil body separates from the plasmosome. What seems to be an early stage in the formation of this body is shown in fig. 12, Pl. 10, taken from a late oocyte where a vacuolated mass can be seen originating from the oxyphil nucleolus. That this process may occur at a much earlier stage is shown by fig. 11, Pl. 10. The plasmosome and a large oxyphil body are present; a bud in close proximity to the latter suggests that these oxyphil emissions have two modes of origin—directly from the plasmosome and from the vacuolated body, the vacuoles being in all probability stages of budding. Although the basophil nucleolus persists as a faintly staining body usually free from granules, the basophil emissions appear to cease after yolk-formation has commenced. At the same time the activity of the oxyphil nucleolus increases, oxyphil bodies being budded off into the nucleus (fig. 13, Pl. 10).

In some cases the activity is so great that the plasmosome appears to be breaking up. The oxyphil emissions spread out in the nucleoplasm and move towards the nuclear membrane, but as in the case of the basophil buds, they were not observed to pass through it. In some cases similar bodies were observed in the ooplasm, these, however, also resembled the smaller yolk-globules.

Some oocytes of the sister species *Thrinax mixta* stained in iron haematoxylin revealed an interesting condition; thus, in fig. 14, Pl. 10, the dark area is apparently the basophil

nucleolus, while the darkly staining small bodies are buds. The other body is the plasmosome, or possibly, the oxyphil vacuolated mass. In fig. 15, Pl. 10, are shown the basonucleolus and oxyphil nucleolus, the latter budding.

The occurrence in this species of what appears to be a closely similar process to that of *Thrinax macula* is worthy of note. The former species was dealt with in a recent paper on saw-fly oogenesis (20), but the present contribution, however, throws new light on some of the phenomena then observed. The exact condition shown in figs. 14 and 15, Pl. 10, was not observed during the previous work.

In the early *Allantus* oocytes, which have not yet become separated from the adjoining nurse-cells, the nucleoli are basophil (fig. 16, Pl. 10). Later, they become more faintly basophil and finally oxyphil. During these changes the nucleolus does not divide into two parts, but changes as a whole from basophil to oxyphil. In the fully formed oocytes before yolk-formation the nucleoli are oxyphil and in the greater number of cases show no traces of basophil nucleoli; but in a certain few an examination revealed the presence of a slightly basophil body, or bodies, containing darkly staining granules (fig. 19, Pl. 10). Thus it would appear that no large basonucleolus is present but that the basophil material is represented by these small bodies. It is difficult to explain why the latter were only shown in certain of the oocytes. They were observed in oocytes during and after yolk-formation (figs. 19 and 20, Pl. 10); the presence of more than one suggests budding having taken place, but no such process was observed in this species. An oocyte in the later stages of yolk-formation revealed a very interesting condition: slightly basophil bodies, without granules, were present in the nucleus and a closely similar body lay outside the nuclear membrane, the appearance and staining reaction of the body being different from that of the yolk-globules (fig. 21, Pl. 10). This is the only case in which a body resembling the basophil bodies of the nucleoli was observed outside the nuclear membrane. It is of interest to note that in most of these bodies observed in the older oocytes, granules were not present.

In the fully formed oocyte, before the formation of yolk, the oxyphil nucleolus enters upon a period of activity during which it gives rise to a number of buds (fig. 19, Pl. 10). This process continues during yolk-formation (fig. 17, pl. 10) and in the later stages is usually more marked and presents the appearance of the nucleolus breaking up. In a few cases a similar condition was noted in earlier oocytes (fig. 18, Pl. 10).

These oxyphil buds pass out towards the nuclear membrane, where they may be seen in close contact with its inner surface, the membrane becoming pushed out at the point of contact. However, they were not observed to pass through the nuclear membrane.

Although many small bodies were shown outside the nucleus, it cannot be said with certainty that they were of nucleolar origin, as in this material they also closely resembled the smaller yolk-globules; but owing to the appearance of other material, and to the evidence of previous work (20), there seems but little doubt that these bodies pass into the ooplasm, where, apparently, they become indistinguishable from the smaller yolk-spheres. Whether the buds subsequently disappear, or are converted directly into yolk, has not been determined.

5. DISCUSSION.

In a recent paper (Peacock and Gresson, 20) a process of nucleolar budding was described in three species of saw-flies. In *Allantus pallipes* the buds were described as occurring outside the nuclear membrane and it was considered that they might give rise to secondary or accessory nuclei. The present contribution does not substantiate this possibility for *Thrinax macula* and *Allantus pallipes*; there is no evidence that accessory nuclei are derived from either the basophil or oxyphil buds of the former species, or from the oxyphil buds and basophil bodies of the latter. But it would seem probable that one or both kinds of buds play some part in yolk-formation, nucleolar activity commencing shortly before the appearance of the yolk-globules.

As previously stated, nucleolar emissions take place in several

groups and in many cases are described as taking part in yolk-formation. Owing to the migration of the oxyphil buds of *Thrinax macula* and *Allantus pallipes* towards the nuclear membrane, and the manner in which they become applied to its inner surface, it seems probable that they are extruded into the ooplasm in a similar manner to that described by Ludford (12) for *Patella*.

These buds may be converted directly into yolk, in which case they would be difficult to differentiate from the small yolk-globules; or they may disappear as described for *Lithobius forficatus* (9). It is of interest to note in this connexion that certain small bodies in the cytoplasm resembled both the smaller yolk-spheres and the nucleolar emissions.

The part played by the basophil buds is more difficult to determine; in *Thrinax macula* they seem to disappear before the yolk is fully formed, while in *Allantus pallipes*, although the basonucleolus was not shown, basophil bodies were observed in certain oocytes during the later stages of yolk-formation. It is worthy of note that in some other forms a basophil nucleolus is not present in the older oocytes. Thus Harvey (6), for *Lumbricus terrestris*, states that a basophil nucleolus occurs in the early oogonia, and later, a plasmosome. In the older oocytes, however, the former has disappeared while the oxyphil nucleolus is still present.

As previously stated, Nath (17) records a change in the staining reactions of the basophil part of the nucleolus of *Culex fatigans*, and more recently (19) describes a change from basophil to oxyphil in spider oogenesis. This change is similar to that of *Allantus pallipes*, except that in the latter the oxyphil nucleolus does not bud off another nucleolus, and in the former 'nucleolar extrusions' do not occur.

Wilson (22) points out that the staining reactions of the nucleoli 'often vary materially at different periods in the history of the nucleus, so that the same nucleolus may be at one time oxyphilic and at another time basophilic'. Thus it would seem, in *Allantus pallipes* the nucleolus changes from basophil to oxyphil, while, at the same time, some of the original baso-

philic material is liberated as small granule-containing bodies. It should be remembered that these bodies were not observed in every oocyte, so that, although they were present in certain of the older eggs, this may be an exceptional condition, their early disappearance being the more general occurrence.

The basophil buds of *Thrinax macula* and the basophil bodies of *Allantus pallipes* are both derived from the basonucleolus, and their behaviour in the nucleus indicates that their ultimate fate is closely similar. In the latter species, however, they are present for a longer period than in the former.

The appearance of a basophil body outside the nuclear membrane of an oocyte of *Allantus pallipes* points to their passing through the membrane, and the fact that this body and others in some of the later oocyte nuclei contained no granules, would seem to suggest that the latter become dissolved, the remainder of the extrusion then being utilized by the ooplasm.

In *Thrinax macula* the basonucleoli present in the oocyte after yolk-formation contain only faintly staining granules, or none at all.

As already stated, Nath (18) describes 'deeply basophil-bodies' as originating from the oocyte nucleoli of *Euscorpius napolii* and *Buthus judacius*; these pass into the cytoplasm, become acidophil and disappear as whole bodies. If the basophil emissions described in the present paper undergo a similar change, it would render their detection in the ooplasm during all stages of yolk-formation very difficult.

The basophil buds of *Thrinax macula* bear a certain resemblance to the accessory nuclei of Buchner (1) and others, but as such were not observed in the ooplasm, it seems more likely that these buds undergo some change and take part in yolk-formation.

The variation in the nucleolar phenomena within these two related species is not an isolated instance; thus, in insects, McGill (14) states that the behaviour of the nucleoli of *Platthemis lydia* differs from that of *Anax junius*; while it is worthy of note that Buchner (1) finds the method of origin of accessory nuclei to vary in different species of saw-flies.

A variation also occurs in the behaviour of the nucleolus in scorpions (18), while Jörgensen (Ludford, 12) and Ludford (12), working on two species of *Patella*, record a difference in the behaviour of the nucleolar emissions.

6. SUMMARY.

1. The material has been obtained from parthenogenetic females. In *Thrinax macula* at least two kinds of females exist, one male-producing, the other female-producing. In *Allantus pallipes* males have not been found.

2. In the early oocytes of *Thrinax macula* the nucleoli are basophil; as they increase in size they develop an oxyphil margin. Later, the oxyphil part becomes rounded off and separates from the basophil. The basophil nucleolus now consists of a small basophil body surrounded by a basophil or slightly oxyphil portion. Vacuoles appear in the outer part, and become larger, in some cases containing dark granules which probably originate from the darkly staining body. The granules increase in size and ultimately become liberated as separate bodies, consisting of a basophil part surrounded by more faintly staining material. These buds may also originate from large vacuolated masses given off from the basophil nucleolus. The buds pass towards the periphery, but were not observed in the ooplasm or passing through the nuclear membrane. They apparently disappear after yolk-formation has commenced; the basonucleolus persists but seems to lose its granules.

3. As the basophil buds are being formed the oxyphil nucleolus enters upon a period of activity, numerous oxyphil buds being liberated.

In some cases the oxyphil buds originate from a vacuolated mass as large as the oxyphil nucleolus, and which arose, probably by constriction, from the latter. Oxyphil buds were observed to migrate towards the nuclear membrane.

4. In *Allantus pallipes* the nucleoli of the early oocytes are basophil; later, they stain more faintly and finally become oxyphil. In the fully formed oocyte before yolk-formation the basophil material is only represented by small bodies containing

dark granules. These bodies may be present during the later stages of yolk-formation, when in some cases they occur as basophil bodies without any granules. In one instance a similar body was observed outside the nuclear membrane.

The oxyphil nucleolus becomes active before yolk-formation commences; it becomes more marked in the later stages and in many cases the nucleolus appeared to be breaking up. The buds occurred in close contact with the inner surface of the nuclear membrane and, later, somewhat similar bodies were observed in the ooplasm; the latter, however, were difficult to differentiate from the smaller yolk-globules.

5. The origin and behaviour of the oxyphil emissions are similar in both species. The oxyphil buds apparently pass into the ooplasm and are utilized during yolk-formation. The basophil buds of *Thrinax macula* and the basophil bodies of *Allantus pallipes* originate from the basonucleolus. The occurrence of basophil bodies without granules in older oocytes, and the presence of one body in one case outside the nuclear membrane of an *Allantus* oocyte suggest that these bodies lose their granules, and are then extruded into the ooplasm, where they play some part in the nourishment of the oocyte.

7. CONCLUSIONS.

1. Oxyphil nucleolar emissions have been found in two Tenthredinid species of two different genera. In origin the emissions are buds from the oxyphil nucleolus, and in *Thrinax macula* also from a large oxyphil body; their fate appears to be extrusion to the ooplasm.

2. Basophil material has also been found in both species, but whereas in *Thrinax macula* it originates by budding from the basophil nucleolus, in *Allantus pallipes* it is found as spherical bodies residual after the transformation of the early basophil nucleolus into an oxyphil nucleolus. Regarding the fate of these buds and bodies everything points to their being extruded into the ooplasm.

3. These two kinds of nucleolar emissions are elaborated practically simultaneously in *Thrinax macula*, but in

Allantus pallipes their presence together has only been detected, so far, after the original basophil nucleolus has become transformed into an oxyphil one.

4. These observations on oxyphil and basophil nucleolar emissions, produced in the manner described under 3, appear novel in the study of insect oogenesis at least, and their nearest parallel appears to exist in the mollusc *Patella*.

8. ACKNOWLEDGEMENTS.

I wish to express my thanks to Professor A. D. Peacock, in whose department this work was carried out, for research facilities, for advice during the course of my investigations, and for supplying me with saw-flies from which my material was obtained.

ADDENDUM.

Since this paper went to press some further contributions to the study of yolk-formation in the invertebrates have appeared. For the chilopod *Otostigmus feae*, Nath ('Quart. Journ. Micr. Sci.', vol. 72) points out that the nucleolar extrusions are few in number, they 'disappear long before the albuminous yolk puts in its appearance in the cytoplasm'; he concludes that they do not give rise to yolk.

On the other hand, Harvey believes that in *Carsinus moenas* ('Trans. Roy. Soc. Edin.', vol. 56, 1929) material is extruded from the plasmosome and subsequently takes part in yolk-formation; while Nath, describing the egg of the fire-fly, *Luciola gorhami* ('Quart. Journ. Micr. Sci.', vol. 73, 1929), states that the albuminous yolk arises from nucleolar extrusions. Thus the condition described in *Carsinus* and in *Luciola* adds further evidence in support of the view that nucleolar extrusions take part in yolk-formation.

REFERENCES.

1. Buchner, P.—"Die akzessorischen des Hymenopteren-ees", 'Archiv. f. Mikr. Anat.', Bd. 91, 1918.
2. Enslin.—"Die Tenthredinoidea Mitteleuropas", 'Deutsche Entom. Zeitschr.', Heft 7, 1912.

3. Gardiner, M. S.—“Oogenesis in *Limulus polyphemus*, with Special Reference to the Behaviour of the Nucleolus”, ‘Journ. Morph. and Physiol.’, vol. 44, 1927.
4. Gatenby, J. B.—“The Cytoplasmic Inclusions of the Germ-cells. Part VI. On the Origin and Probable Constitution of the Germ-cell Determinant of *Apanteles glomeratus*, with a Note on the Secondary Nuclei”, ‘Quart. Journ. Micr. Sci.’, vol. 64, 1920.
5. ——— “Part X. The Gametogenesis of *Saccocirrus*”, *ibid.*, vol. 66, 1922.
6. Harvey, L. A.—“On the Relation of the Mitochondria and Golgi Apparatus to Yolk-formation in the Egg-cells of the Common Earth-worm, *Lumbricus terrestris*”, *ibid.*, vol. 69, 1925.
7. Hogben, L. T.—“Studies on Synapsis. I.—Oogenesis in the Hymenoptera”, ‘Proc. Roy. Soc.’, B, vol. 91, 1920.
8. ——— “Studies on Synapsis. II. Parallel Conjugation and the Prophase Complex in *Periplaneta* with Special Reference to the Pre-meiotic Telophase”, *ibid.*
9. King, S. D.—“Oogenesis in *Lithobius forficatus*”, ‘Scient. Proc. Roy. Dublin Soc.’, vol. 18, no. 3, 1924.
10. ——— “Note on the Oogenesis of *Peripatopsis Capensis* (Purcell)”, ‘Quart. Journ. Micr. Sci.’, vol. 70, 1926.
11. Ludford, R. J.—“Contribution to the Study of the Oogenesis of *Patella*”, ‘Journ. Roy. Micr. Soc.’, 1921.
12. ——— “The Behaviour of the Nucleolus during Oogenesis, with Special Reference to the Mollusc *Patella*”, *ibid.*
13. ——— “The Morphology and Physiology of the Nucleolus. Part I. The Nucleolus in the Germ-cell Cycle of the Mollusc *Limnaea stagnalis*”, *ibid.*, 1922.
14. McGill, C.—“The Behaviour of the Nucleoli during Oogenesis of the Dragon-fly, with Special Reference to Synapsis”, ‘Zool. Jahrb. Jena, Abt. für Anat.’, Bd. 23, 1906.
15. Nakahara, W.—“On the Physiology of the Nucleoli as seen in the Silk-gland Cells of certain Insects”, ‘Journ. Morph.’, vol. 29, 1917.
16. Nath, V.—“Oogenesis of *Lithobius forficatus*”, ‘Proc. Camb. Phil. Soc. Biol. Sci.’, vol. 1, no. 3, 1924.
17. ——— “Egg-follicle of *Culex*”, ‘Quart. Journ. Micr. Sci.’, vol. 69, 1924.
18. ——— “Cell Inclusions in the Oogenesis of Scorpions”, ‘Proc. Roy. Soc.’, B, vol. 98, 1925.
19. ——— “Studies in the Origin of Yolk. I. Oogenesis of the Spider”, ‘Quart. Journ. Micr. Sci.’, vol. 72, no. 2, 1928.
20. Peacock, A. D., and Gresson, R. A. R.—“The Roles of the Nurse-cells, Oocytes, and Follicle cells in Tenthredinid Oogenesis”, *ibid.*, vol. 71, 1928.

21. Peacock, A. D.—'Journ. Scient. Trans.', British Association for the Advancement of Science, pp. 29-30, 1928.
22. Wilson, E. B.—'The Cell in Development and Heredity', pp. 91-6, 1925. New York.

EXPLANATION OF PLATE 10.

The drawings were made by means of a Zeiss camera lucida and a Watson 'Service' Microscope. For figs. 1-16 a Leitz $\frac{1}{12}$ objective was used, and for all others a Reichert $\frac{1}{12}$. The eyepiece was a Hawksley no. 4 \times 10.

LETTERING.

b b, basophil body; *b g*, basophil granule; *b n*, basophil nucleolus; *b n b*, basophil nucleolar bud; *f c*, follicle cell; *l b g*, large basophil granule in basophil nucleolus; *nu*, early nucleolus; *o b*, oxyphil body; *o n*, oxyphil nucleolus; *o n b*, oxyphil nucleolar bud; *sp*, space between follicle wall and nucleus; *y*, yolk.

PLATE 10.

All figs. from *Thrinax macula*; figs. 5, 6, 7, and 11 from adults, all others from pupae.

Figs. 12 and 13 from adult of *Thrinax macula*; figs. 14 and 15 from *Thrinax mixta* adult. All other figs. from pupae of *Allantus pallipes*. The darkly shaded areas in figs. 12, 14, and 15, and in figs. 17-22 represent more darkly stained parts of the nucleus; this appearance may be due to the action of the fixative.

Fig. 1.—Early oocyte, showing basophil nucleolus.

Fig. 2.—Later stage showing the oxyphil nucleolus arising from the basophil part.

Fig. 3.—Later oocyte which has become completely separated from the nurse-cells; the oxyphil and basophil parts of the nucleolus are distinct.

Fig. 4.—Oocyte before yolk-formation; oxyphil and basophil nucleolus; the basophil part consists of darkly staining granule surrounded by lighter part.

Fig. 5.—Later oocyte showing vacuoles, some of which contain dark granules in basophil nucleolus.

Fig. 6.—Oocyte before yolk-formation. Oxyphil nucleolus giving rise to buds; granules scattered through basophil nucleolus.

Fig. 7.—Oocyte before yolk-formation. Oxyphil and basophil nucleolus giving rise to buds.

Fig. 8.—Oocyte before yolk-formation. Oxyphil nucleolus giving rise to buds; basophil buds are shown in the ooplasm; the large vacuolated body with dark granules has probably been given off from the basophil nucleolus.

Fig. 9.—Later oocyte, showing basophil buds in the ooplasm. The oxyphil nucleolus is not shown in this section.

Fig. 10.—Oocyte before yolk-formation. Showing basophil buds close to the nuclear membrane. Oxyphil and basophil nucleolus not shown.

Fig. 11.—Oocyte before yolk-formation. Showing oxyphil and basophil nucleolus; the large vacuolated body present is probably given off from the oxyphil.

Fig. 12.—From late oocyte after formation of yolk. Showing a stage in the liberation of the vacuolated body from the oxyphil nucleolus. Basophil nucleolus not shown.

Fig. 13.—Late oocyte after yolk-formation. The oxyphil nucleolus is giving rise to buds, the basophil nucleolus is not budding.

Fig. 14.—From oocyte before yolk-formation. The dark area is apparently the basophil nucleolus, it is giving rise to buds. The oxyphil nucleolus is shown.

Fig. 15.—From oocyte before yolk-formation. Showing oxyphil and basophil nucleolus.

Fig. 16.—Early oocyte before complete separation from nurse-cells. The nucleolus is basophil.

Fig. 17.—Early stages of yolk-formation. Showing oxyphil nucleolus giving rise to buds.

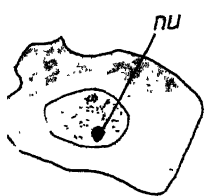
Fig. 18.—From oocyte before formation of yolk. Oxyphil nucleolus breaking up into buds.

Fig. 19.—Oocyte before yolk-formation. Oxyphil nucleolus budding; basophil bodies containing dark granules present.

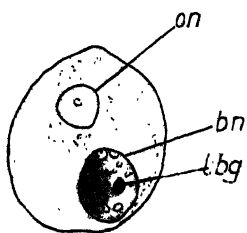
Fig. 20.—Oocyte during yolk-formation. Oxyphil buds and basophil bodies present.

Fig. 21.—From oocyte during yolk-formation. Showing basophil bodies in the nucleus, a closely similar body is shown outside the nuclear membrane.

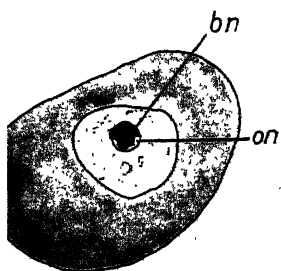
Fig. 22.—From oocyte before yolk-formation. Showing oxyphil buds closely applied to the inside of the nuclear membrane.



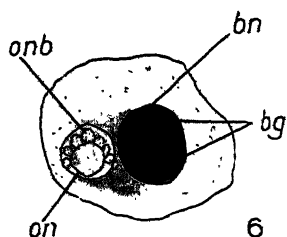
1



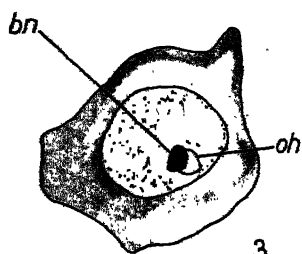
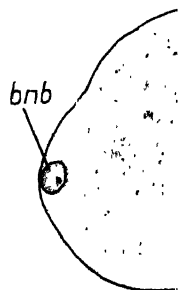
5



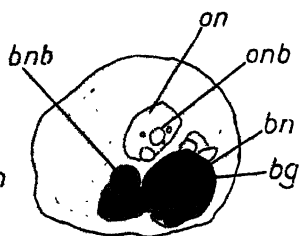
2



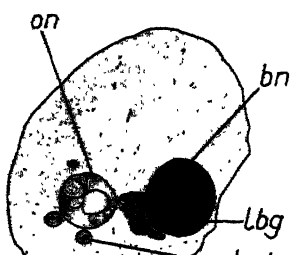
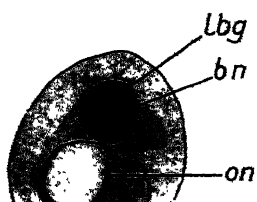
6

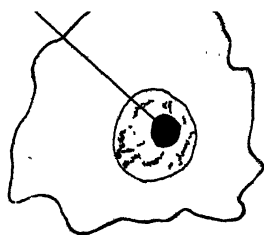


3



7

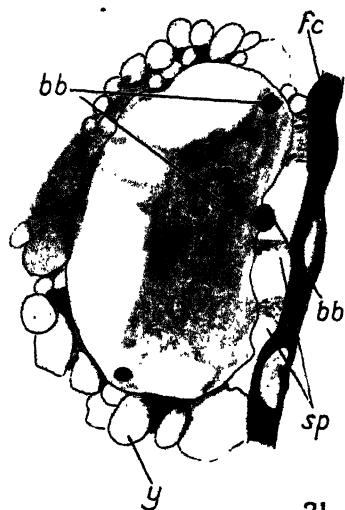




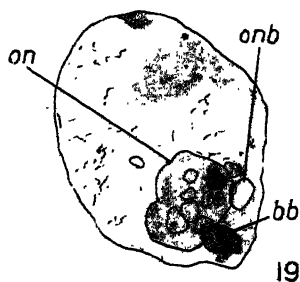
16



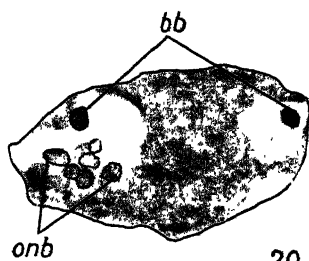
18



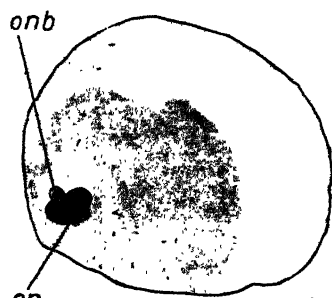
21



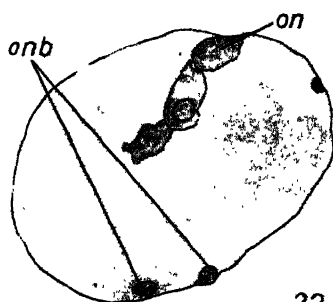
19



20



23



22

Scale
0.01 mm



Nucleolar behaviour in the Mitosis of Plant Cells.

By

Priscilla E. Frew and Robert H. Bowen,

Department of Zoology, Columbia University.

With Plate 11.

INTRODUCTION.

THE process of mitosis is one of the most impressive and intriguing of biological phenomena. Since its discovery it has been responsible for an increasing amount of speculation as to its nature and exact significance. With the development of the chromosomal theory of heredity the intent of so elaborate a procedure has become clear, but we are still in almost complete ignorance of the dynamic factors involved in most of its phases. Theories as to the exact nature of these forces are very numerous, but no one of them seems adequate to explain more than a limited number of the facts.

It has become clearly recognized from a mass of observation and experiment that the apparent continuity of the whole process of mitosis is the result of a remarkable synchronization of a number of different phases, each one of which must probably be explained independently. One of these phases that is largely independent of the others is the anaphasic movement of the chromosomes. This paper is chiefly concerned with observations¹ that seem to have some significance in relation to theories of the factors involved in bringing about the poleward movement of chromosomes.

¹ The major facts described in this paper were first tentatively made out during the course of an extended study of plant material with another purpose in view. A subsequent prolonged search through the literature of plant cytology has brought to light a considerable number of apparently similar cases among angiosperms, in none of which, however, have the phenomena been very completely described.

MATERIAL AND METHODS.

The material for this study comprised members of the genus *Cucurbita*.¹ Root-tips of the Hubbard squash variety of *Cucurbita maxima*, and of the Connecticut Field, Winter Luxury, and English Vegetable Marrow varieties of the pumpkin, *Cucurbita pepo*, were prepared for study. The seeds were sprouted on wet filter-paper in moist chambers, and the root-tips were cut off with a razor and fixed, when 2-3 mm. in length.

The following fixatives were used : Mottier, Bouin, Hermann, strong and weak Flemming, vom Rath, and a saturated aqueous solution of corrosive sublimate. Fixation in Mottier's fluid was followed by the regular Benda method of mordanting and staining. Auerbach's acid fuchsin-methyl green combination was used on sections of root-tips that had been fixed in the sublimate solution. After all the other fixing fluids either Fe-haematoxylin or safranin was used as a basic dye. This was sometimes followed by counterstaining with light green or eosin. Both longitudinal and cross-sections were cut at thicknesses of 4-6 micra.

OBSERVATIONS.

A. The Resting Nucleus.

The cells of the meristematic region of the root-tip of both species of *Cucurbita* studied have relatively large nuclei, the greater part of the contents of which consists of a relatively huge, spherical nucleolus² (fig. 1, Pl. 11). In the sectioned and stained material this nucleolus is commonly surrounded by a clear, unstained area, which probably is a shrinkage artifact. The nucleolus stains heavily with haematoxylin and safranin. With Auerbach's acid fuchsin-methyl green combination it is stained red ; with Benda it is a brick-red colour. Outside the

¹ We have from time to time also noted stages indicative of phenomena similar to those here to be described in *Cucurbita* in several other angiosperms (and, with some modifications of detail, in *Equisetum*).

² In root-tip cells of many plants the occurrence of an unusually large amount of nucleolar material is a striking characteristic. This may be concentrated in one very large nucleolus, as in *Cucurbita*, or distributed in several distinct masses, as in *Equisetum*, &c.

nucleolus there is a small region containing scattered chromatin granules. The identification of this material as chromatin is based particularly upon the fact that it is stained green by the Auerbach combination. The granules are very small, and it was not possible to determine their exact relation to a general nuclear reticulum. Except for these granules the region between the nucleolus and the nuclear membrane appears rather homogeneous.

B. The Prophase.

In *Cucurbita maxima*, prior to the breakdown of the nuclear membrane in the late prophase, there is formed a coarse spireme of fairly uniform diameter (fig. 2, Pl. 11). The relation of this spireme to the scattered chromatin granules seen in the resting nucleus is obscure, the small size of the cells making it difficult to distinguish early stages in the spireme development. In *Cucurbita pepo* we have been unable to find a similar spireme. The formation of the spireme in *Cucurbita maxima* appears to be accompanied by no marked change in the nucleolus beyond a possible slight decrease in size. Either contemporary with, or prior to, the disappearance of the nuclear membrane the spireme apparently becomes broken up into a number of small, spherical or cylindrical chromosomes, clustered on the periphery of the nucleolus, which is now irregular in shape (fig. 3, Pl. 11). By the time the spindle has developed and the chromosomes have moved to their equatorial position upon it the nucleolar material has in many cases entirely disappeared, as, indeed, is customary in mitosis in both animal and plant cells. In some cases,¹ however, the nucleolus behaves as though its material was relatively too abundant for dissolution to be completed during the prophase period.² It then persists throughout the entire prophase period and is caught in the centre of the spindle-area, becoming elongated to form a cylinder with its long axis parallel to that of the developing spindle (fig. 4, Pl. 11). This alteration in shape is significant in view of the metaphase phenomena to be described below.

¹ Particularly in the outer layers of the plerome.

² See also Tischler (1922).

C. The Metaphase.

Nucleolar material thus frequently persists up to the beginning of the metaphase. In such cases, in the early metaphase the nucleolus, in the form roughly of a cylinder, is always found in the centre of the equatorial plate of chromosomes, with its long axis parallel to that of the spindle (figs. 5, 10, and 11, Pl. 11). Polar views (figs. 10 and 11, Pl. 11) emphasize the fact that the nucleolus is actually within the spindle region and surrounded by a broad ring of chromosomes. Perhaps a better description would be that the nucleolus 'perforates' the plate of chromosomes more or less centrally, being often much contracted at the point of perforation (fig. 10, Pl. 11), and enlarged at both extremities. This orientation is not always symmetrical with respect to the equatorial plate, since it frequently happens that the chromosomes surround the nucleolus in a plane much closer to one end of the cylindrical nucleolus than the other.

The nucleolus next becomes drawn out to a shape usually resembling a dumb-bell (figs. 6, 7, and 8, Pl. 11). Occasionally nearly all of the cylinder lies in one-half of the spindle, and in such a case the nucleolus becomes drawn out to a shape shown in fig. 9, Pl. 11. In either case, the two ends of the nucleolus continue their movement towards opposite spindle-poles with a consequent stretching of the part in the equatorial region, until finally the strand connecting the two polar portions is ruptured (figs. 13 and 14, Pl. 11). The two masses of nucleolar material thus formed continue their poleward migration and eventually reach the poles of the spindle (figs. 12, 15, and 16, Pl. 11). As would be expected from the variation in orientation of the nucleolus before and during its division, the two spheres may be nearly equal in size (fig. 12, Pl. 11), or markedly unequal as in fig. 15, Pl. 11. Sometimes the size differences are even more pronounced. When the nucleolar fragments are approximately equal, as in fig. 16, Pl. 11, their simulation of the centrioles in some animal-cells is rather striking. During all this time the nucleolus appears to be undergoing a progressive shrinkage in size, although positive determination of this fact is difficult

because of initial variations in nucleolar size at the close of the prophase. However, we infer that there is a shrinkage from the fact that the sum of the volumes of the largest pair of spheres observed in the polar position never appears to be as great as that of the larger nucleoli before metaphase. During the metaphase phenomena the nucleolus is commonly seen in fixed and stained preparations to be surrounded by a narrow clear zone. One is tempted to assume that this represents a region where dissolution of the nucleolus, resulting in a reduction of its size, is taking place, but possibly it is an 'artifact resulting from shrinkage, like that seen in the resting nucleus.

D. The Anaphase.

As is usually the case, the anaphase migration of the chromosomes in *Cucurbita* appears to be a very rapid process, and intermediate stages of it are very rare. Apparently no significant change takes place in the nucleolar masses, and, being slightly pushed away from their exact polar locations, they are seen at the end of the anaphase lying adjacent to the chromosome plates and appearing very much as at the close of their metaphase migration (fig. 17, Pl. 11).

E. The Telophase.

During the telophase reconstruction of the nucleus the nucleolar masses continue to remain clearly apart from the chromosome groups, although they may be in their near vicinity (figs. 18 and 19, Pl. 11). The shrinkage process apparently continues (fig. 19, Pl. 11). During the nuclear reconstruction a new mass of nucleolar material appears in the midst of each group of chromosomes. It has no apparent connexion with the old nucleolar mass although the occasional close juxtaposition of the chromosome plates and these old nucleolar fragments sometimes makes this point difficult to determine. But in cases where the polar movement has proceeded to such an extent as in fig. 17, Pl. 11, it would seem practically certain that an intimate topographical relationship could never occur without a further movement of either the chromosomes or the

nucleolar mass, and for such movements there is no evidence whatsoever. All the facts indicate that during the reorganization of the daughter nuclei the nucleolar fragments of the parent cell remain entirely extranuclear, and ultimately undergo complete disintegration in the polar cytoplasm. In sister cells in which the nuclei have been completely reconstructed it has not been possible to identify any nucleolar material in the cytoplasm. Small granules are occasionally seen, but they cannot certainly be distinguished from cytoplasmic granules that never had any relation to the nucleolus. Probably by this stage the dissolution of the nucleolar remnants has proceeded to such an extent that they have either disappeared entirely or shrunk to insignificant and unrecognizably small masses. We wish to be very emphatic upon one point, namely, that, in spite of the simulation of chromosome behaviour shown by the equatorial orientation and subsequent division of the nucleolus, the evidence practically demonstrates that this is merely a necessary result of the more or less accidental catching of the nucleolus in the spindle-region. The nucleolus is strikingly different from the chromosomes in that there is no direct continuity of its substance from one cell-generation to another.

DISCUSSION.

In this study our attention has been chiefly directed toward the nucleolar phenomena, and observations of other structures in the cell have been entirely incidental. This discussion will be largely confined to the same topic, namely, the behaviour of the nucleolus during mitotic division.

A resting nucleus of the same general character as that in *Cucurbita* has been described in *Azolla* by de Litardière (1921). The similarity centres largely in the presence of numerous small granules of chromatin of fairly uniform size. In the case of *Azolla* the evidence strongly suggests that these granules are chromosomes which are persistent throughout the interkinetic phase. These chromosomes appear to go on the metaphase plate without ever forming a definite spireme. Our observations indicate the possibility of similar phenomena in

Cucurbita pepo, but positive determination of this fact awaits more careful investigation with this particular object in mind.

The nucleolus is usually described as approximately spherical in resting plant-nuclei, and the clear space around it is commonly shown in published figures. Our tentative conclusion that this clear area is largely an artifact—the result of shrinkage—is based in part on the statement of Lundegårdh (1912) that in living cells such a space is very seldom observed, even in those forms which show it most clearly in the resting nuclei of fixed preparations.

The irregularity in shape of the prophase nucleolus has also been frequently described. Nucleoli have been observed to undergo amoeboid changes of form during the prophase in living cells of *Chara* (Zacharias, 1902) and *Vicia* (Lundegårdh, 1912). We have no clue to any broad significance which may attach to this phenomenon and it probably merely indicates some change in the physical character of the nucleolus or nuclear sap that is incidental to the prophase condition in the nucleus as a whole. The spherical form of the resting nucleolus is assumed by Lundegårdh to indicate a very fluid consistency. If this be true, the prophasic amoeboid form might be construed as an indication either of an increase in viscosity or of a decrease in surface tension.

During the prophase of cell division the nucleolus commonly undergoes a progressive shrinkage, which has often been emphasized because of its supposed indication of the transfer of material from the nucleolus to the growing chromosomes. This shrinkage commonly results in the disappearance of the nucleolar material in the earlier prophase, or at the latest before the breakdown of the nuclear membrane, as in *Allium*, &c. In other cases, however, the nucleolar substance may persist into the late prophase and thus be cast out into the cytoplasm at the breakdown of the nuclear membrane, as in *Pustularia* (Bagchee, 1925), where the final stages in nucleolar dissolution take place in the cytoplasm adjacent to the equatorial region of the spindle. Finally, in some cases, the persistent nucleolus

may be caught in the spindle and involved in the actual division processes, as in *Cucurbita*. The fact that the decrease of nucleolar mass progresses throughout the anaphase and telophase stages in the last-mentioned cases seems to us significant in view of the hypothesis occasionally put forward that the prophase shrinkage of the nucleolus indicates some direct contribution from the nucleolus to the concomitant increase in the chromatin. Certainly chromosome growth usually ceases in the late prophase, yet the nucleolar shrinkage continues, while the nucleolar remnants become widely separated from the chromosomes (fig. 14, Pl. 11). This whole series of events seems to indicate that we have to do here with two coincident but otherwise unrelated processes, namely, increase of chromatin and decrease of nucleolar substance. The nucleolus disintegrates, not because it contributes to chromatin development, but more probably as a result of the new physical or chemical conditions in the nuclear sap—conditions that are probably incidental to the mitotic process as a whole.

The division of what superficially at least resembles nucleolar material,¹ and the polar migration of the division products coincident with or subsequent to the actual separation of the chromosomes has been described in a variety of forms. An example of such a process in the Protista is the case of *Euglena* (Keuten, 1895; Tschenzoff, 1916, and others). It has been described among the filamentous green Algae by Berghs (1906) in *Spirogyra*, by Němec (1910*a*) in *Cladophora simplicior*, and by de T'Serclaes (1922) in *Cladophora glomerata*. It has been noted also in the myxomycete *Spongiospora* by Osborn (1911), and in the Plasmodiophorales by Cook (1928). One example has been reported in the pteridophytes—the case of *Marsilia* (Berghs, 1907) where the nucleolus is caught in the spindle, but dissolves during the metaphase. Many cases of this sort have also been more or less completely reported in the higher plants. It

¹ Although the general opinion seems to be that in lower forms this material is not actually equivalent to the true nucleolus of the higher plant-cell.

has been unmistakably described in *Phaseolus* by Rosen (1896), in *Roripa* by Němec (1897), in *Solanum* by Němec (1899), in *Alnus* and *Hibiscus* by Němec (1901*a* and *b*), in *Phaseolus* by Wager (1904) and Martins Mano (1905), in *Ricinus* and *Cucurbita maxima* by Němec (1910*b*), in *Cucurbita pepo* by Lundegårdh (1912), in *Lupinus* by de Smet (1914), in *Helianthus* by Tahara (1915), in *Clivia* by Van Camp (1924), and in *Canna* and *Lupinus* by Schaede (1928).¹ In all these cases in the higher plants it appears that nucleolar material sometimes persists until the metaphase, in which case it is caught in the spindle, and is probably divided essentially as we have described in *Cucurbita*. In the lower plants the division of nucleolar material usually accompanies the separation of the chromosomes instead of preceding it, and not infrequently the nucleolar derivatives are ultimately incorporated in the daughter nuclei as the definitive nucleolus. In most of the cases mentioned above among angiosperms the original descriptions are limited to a few sentences and but two or three figures. The work of Van Camp (1924) is distinguished by his more positive identification of the nucleolar material by means of staining with the Auerbach combination. Both Lundegårdh's and Němec's descriptions of *Cucurbita* are limited to a few figures and casual references in the text, since the main purpose of their studies lay in another direction. Van Camp's paper is fairly complete and includes some review of earlier work.

Apparently Němec at least clearly appreciated the significance of these observations in relation to general theories of the factors responsible for the anaphase movement of the chromosomes. At the time of his paper (1901) the most widely accepted hypothesis was that of Van Beneden and others that the chromosomes were pulled to the poles by the contraction of the

¹ There are also cases in which the nucleus characteristically contains more than one nucleolus, the nucleoli being transported intact to the opposite spindle poles during the prophase-metaphase transition—for example, Karsten (1893) in *Psilotum*, and Bargagli-Petrucci (1905) in *Equisetum*. See also Zimmermann (1893) and Lenoir (1926).

attached spindle fibres. In a rather extensive discussion of this view Němec strongly emphasized the point that the division and polar migration of a nucleolus, to which there were certainly no spindle fibres attached, was a practically insurmountable difficulty in the way of explaining anaphase chromosome movement as due to fibrillar contractility. This theory, Němec pointed out, could only be maintained if it assumed that the chromosomes and nucleolar fragments were moved to the poles by different forces.

This same objection to theories of fibrillar contractility was later indicated by Bonnet (1912). This author was more emphatic in his declaration that the division of the nucleolus definitely ruled out the theory of Van Beneden, and he concluded that all that is known of the process of anaphase migration is that there exist in the cell at mitosis certain factors which ordinarily cause the movement of certain cellular constituents towards the poles. He believed that these forces were not necessarily confined to the spindle region, since amyloplasts in the surrounding cytoplasm occasionally participated in the poleward movement. Bonnet was completely sceptical about the role of a spindle in the process, and believed that it had merely a remarkable chronological relation to the other phenomena—an extreme view to which we can hardly subscribe when we consider the universal presence of a spindle in cells undergoing mitosis.

In an instructive discussion of the mechanism of mitosis Tischler (1922) reiterates this argument against the fibrillar contractility theory. This author also considers its relation to the so-called 'Stemmtheorie' originated by Drüner (1895) and recently revived by Bélař (1927). This theory holds that the chromosomes move to the poles because they are pushed apart by the growth of the interzonal fibres as a whole (Stemmkörper) in the region between the daughter chromosomes. As Tischler points out, this theory is quite adequate to explain the polar migration of all the chromosomes simultaneously, but completely fails to allow for a precocious division of nucleolar material. It might be added that the theory also fails to

explain the precocious anaphase migration of some sex chromosomes, and even of some autosomes. These difficulties have recently been considered by Bělař (1928), who suggests means of circumventing them.

It is evident that our observations on *Cucurbita* offer no immediate solution of the problems presented by the mitotic spindle and the anaphase movements of the chromosomes. They do, however, clearly emphasize two features of importance. Firstly, that the anaphase migration of the daughter chromosomes is apparently not directly due to the so-called spindle-fibres with which they are in connexion, since the nucleolar portions behave in exactly the same way without any spindle-fibre attachments whatever; and secondly, that the products of the division of the nucleolus move to the spindle poles while the chromosomes, though already divided, remain unmoved in the equatorial region of the spindle.

With regard to the first of these points, our observations would seem to suggest that the spindle area represents a region in which are localized those forces of whatever kind which are responsible for anaphasic movements. This localization is most strikingly demonstrated in cases like that of *Pustularia*, where the nucleolus is in most instances left outside the spindle at metaphase and disintegrates in the equatorial cytoplasm, but on rare occasions divides as in *Cucurbita* simply because it is by chance caught in the spindle area. It is clear from other work that movements are also on foot in the general cytoplasm looking toward the bipolar orientation of chondriosomes and archiplasts during mitosis, but the identity of these with the factors at work within the spindle area seems to us by no means so clear as Bonnet, for example, assumed.

If the spindle then represents a specialized area within which very definite, directed movements take place, it seems probable that whatever the force at work, it would be equally potent regardless of the nature of the bodies which found themselves in the spindle region—whether chromosomes or nucleoli. Thus, in the present case, the circumstances strongly suggest that the presence of the nucleolus within the spindle region is a pure

matter of chance, dependent primarily on the fact that its disintegration during the prophase has proceeded too slowly. The nucleolus may thus be thought of in terms of some foreign body inserted into the spindle area, and its resulting division and movements depend entirely on just where the nucleolus happens to lie with respect to the mid-region of the spindle. Presumably any other small mass of proper consistency inserted into the spindle would behave in the same way.¹ All these things indicate that the 'something' which moves chromosomes towards the spindle poles is not especially associated with the chromosomes as such. Neither does this 'something' reside in the chromosomes themselves. Rather do the chromosomes move in response to the same forces which would move anything placed in the same position.

It is, then, abundantly clear that the movements of the chromosomes cannot depend on the 'contraction' of spindle-fibres, or any other such special apparatus. Some hypothesis involving protoplasmic streaming could doubtless be suggested, and, indeed, the recent work of Chambers (1917) on protoplasmic currents in the asters of the cleaving egg,² and the studies of Spek (1918) on artificial simulacra of cleavage processes, recall the old suggestion of Bütschli (1876) that chromosome migration may be effected by streaming movements in the spindle; also the view later held by Berthold (1886) that the chromosomes might be pushed apart by the streaming of cytoplasmic material into the mid-plane of the spindle. Unfortunately we have absolutely no definite information concerning the dynamic con-

¹ In this connexion it is of unusual interest to recall the observations of Konopacki (1911) on the behaviour of the nucleus in sea-urchin eggs exposed to hypertonic solutions. The abnormal chromosomes, sometimes even the intact nucleus, having reached a position between the asters, are dragged apart into two portions which eventually reach the spindle poles. The figures given by Konopacki of the division of intact nuclei by such a procedure are remarkably suggestive of the behaviour of the nucleolus in *Cucurbita*.

² Most interesting is the fact that oil droplets on the periphery of an aster will be carried towards the astral centre if they be first guided by a microdissection needle into the inflowing stream (Chambers, 1917).

ditions which prevail in the spindle, and the presence of anything comparable to 'streaming movement' has not been recognized. It seems to us, therefore, at present of doubtful usefulness to attempt to trace the behaviour of bodies in the spindle area to mere protoplasmic currents. The important point is that whatever the nature of the force at work in the spindle area, it is nothing which has to do specifically with the chromosomes. It is rather part and parcel of the whole achromatic division figure.

The difficulty remains that the chromosomes do not move until a definite moment in the mitotic cycle has been reached, in spite of the clear evidence that the factors responsible for their migration are operative relatively early in spindle formation. The reasons for this stability of the equatorial chromosome complex seem to be bound up with the well-known fact that the spindle area is, in part at least, a region of higher viscosity than the surrounding cytoplasm. This is true not only of the metaphase, but particularly of the late anaphase, as Béla's (1927) recent study of living cells has so clearly demonstrated. But concerning any details of the morphological structure of this viscous spindle area, again we must confess an almost complete ignorance. It is, however, difficult to see how the viscosity of the spindle can be directly invoked as an explanation for the retention of the chromosomes in an equatorial position, since the nucleolar fragments are meanwhile moving poleward presumably propelled by the same mechanism which finally moves the chromosomes as well. In this connexion it is perhaps of interest to recall the demonstration recently given by Nassonov (1918) that in plant mitosis the 'fibres' attached to the chromosomes are definitely demonstrable by osmic-acid impregnation, while the so-called central spindle remains unblackened and apparently structureless.¹ If the 'fibres' thus demonstrated represent regions of unusually high protoplasmic viscosity, the metaphase retention of the chromosomes

¹ Nassonov's extended discussion of the structure of the spindle is most interesting in the light of the suggestions made in this paper. His account of the poleward retraction of the chromosomal fibres is particularly noteworthy.

in the equator would receive an obvious explanation. The ultimate movement of the chromosomes could be brought about by the progressive liquefaction of the fibres at one or both ends. The interzonal fibres, or 'Stemmkörper', developed between the diverging chromosomes, represent presumably the formation of a new area of very high viscosity which leaves the spindle in a remarkably firm condition and perhaps in its development assists in the extreme pushing apart of the daughter chromosome groups as a whole—a phenomenon which is indeed well known from the work of several observers, most recently Bělař (1927).

These suggestions are here put forward by way of further approach to a problem which has hitherto proved extraordinarily baffling. The ultimate solution of the structure and dynamics of the 'spindle' clearly demands much further investigation.

SUMMARY.

1. The resting nuclei of *Cucurbita pepo* and *Cucurbita maxima* contain a single, spherical nucleolus that is relatively very large. Part, at least, of this nucleolus persists during nearly the entire process of mitosis. During the metaphase it lies in the equatorial plate and becomes elongated in the direction of the long axis of the spindle, to form first a cylinder and then, with further elongation, a dumb-bell-shaped structure, which finally separates into two fragments that migrate to the opposite poles of the spindle. This entire movement occurs prior to any anaphase migration of the chromosomes. The nucleolar fragments apparently are not included in the daughter nuclei during the telophasic reconstruction, but degenerate in the cytoplasm.

2. Examination of the literature suggests that a similar process of nucleolar division probably occurs in a wide variety of plant-cells.

3. The bearing of this type of nucleolar division on theories of the dynamics of anaphase migration of the chromosomes is briefly discussed.

LITERATURE CITED.

- Bagchee, K. (1925).—"Cytology of the Ascomycetes. I. Spore development", 'Ann. Bot.', vol. 39.
- Bargagli-Petrucci, G. (1905).—"I nucleoli durante la cariocinese nelle cellule meristematiche di *Equisetum arvense*", 'N. Giorn. botan. ital.', N. Ser., tom. 12.
- Bělař, K. (1927).—"Beiträge zur Kenntnis des Mechanismus der indirekten Kernteilung", 'Die Naturwissenschaften', Bd. 15.
- (1928).—"Die cytologischen Grundlagen der Vererbung". Berlin.
- Berghs, J. (1906).—"Le noyau et la cinèse chez le *Spirogyra*", 'La Cellule', tom. 23.
- (1907).—"Les cinèses somatiques dans le *Marsilia*", *ibid.*, tom. 25.
- Berthold, G. (1886).—"Studien über Protoplasmamechanik". Leipzig.
- Bonnet, J. (1912).—"Recherches sur l'évolution des cellules nourricières du pollen", 'Arch. f. Zellforsch.', Bd. 7.
- Bütschli, O. (1876).—"Studien über die ersten Entwicklungsvorgänge der Eizelle, die Zellteilung und die Conjugation der Infusorien", 'Abh. Senck. Nat. Ges. Frankfurt', Bd. x.
- Chambers, R. (1917).—"Microdissection studies. II. The cell aster: a reversible gelation phenomenon", 'Journ. Exp. Zool.', vol. 23.
- Cook, W. R. I. (1928).—"The methods of nuclear division in the *Plasmodiophorales*", 'Ann. Bot.', vol. 48.
- Conklin, E. G. (1912).—"Cell size and nuclear size", 'Journ. Exp. Zool.', vol. 12.
- Drüner, L. (1895).—"Studien über den Mechanismus der Zellteilung", 'Zeitschr. f. Naturwiss.', Bd. 29.
- Karsten, G. (1893).—"Ueber Beziehungen der Nucleolen zu den Centrosomen bei *Psilotum triquetrum*", 'Ber. d. d. bot. Ges.', Bd. 11.
- Keuten, J. (1895).—"Die Kernteilung von *Euglena viridis*", 'Zeitsch. f. wiss. Zool.', Bd. 60.
- Lenoir, M. (1926).—"Biologie de la cellule", 'Arch. de Morph. gén. et exp.', Fasc. 26.
- Litardière, R. de (1921).—"Recherches sur l'élément chromosomique dans la caryocinèse somatique des *Filicinées*", 'La Cellule', tom. 31.
- Lundegårdh, H. (1912).—"Chromosomen, Nucleolen, und die Veränderungen im Protoplasma bei der Karyokinese", 'Beitr. z. Biol. d. Pflanz.', Bd. 11.
- Martins, Mano T. (1905).—"Nucléole et chromosomes dans le méristème racinaire de *Solanum tuberosum* et *Phaseolus vulgaris*", 'La Cellule', tom. 22.
- Nassonov, D. (1918).—"Recherches cytologiques sur les cellules végétales", 'Arch. Russ. d'Anat., Hist., et Embry.', tom. 2.

- Némeç, B. (1897).—"Cytologische Untersuchungen von Vegetationspunkten der Pflanzen" (Bohemian, with German résumé), 'Sitzber. d. kön. böhm. Ges. d. Wiss.', no. 33.
- (1899).—"Ueber Kern- und Zelltheilung bei *Solanum tuberosum*", 'Flora', Bd. 86.
- (1901 a).—"Neue zytologische Untersuchungen. Die Kernteilung in der Wurzelspitze von *Alnus glutinosa*", 'Beitr. z. wiss. Bot.', Bd. 4.
- (1901 b).—"Ueber centrosomenähnliche Gebilde in vegetativen Zellen der Gefäßpflanzen", 'Ber. d. d. Bot. Ges.', Bd. 19.
- (1910 a).—"Ueber die Kernteilung bei *Cladophora*", 'Bull. int. Acad. Sc. Bohême. Cl. d. sc. math.'
- (1910 b).—"Das Problem der Befruchtungsvorgänge". Berlin, 1910.
- Osborn, T. G. B.—"Spongospora subterranea", 'Ann. Bot.', vol. 25.
- Rosen, F. (1896).—"Beiträge zur Kenntnis der Pflanzenzellen", 'Beitr. z. Biol. d. Pflanz.', Bd. 7.
- Schaeede, R. (1928).—"Ueber das Verhalten des Nucleolus während der Kernteilung", 'Protoplasma', Bd. 5.
- de Smet, E. (1914).—"Chromosomes, prochromosomes et nucléole dans quelques dicotylées", 'La Cellule', tom. 29.
- Spek, J. (1918).—"Oberflächenspannungsdifferenzen als eine Ursache der Zellteilung", 'Arch. Entw.-Mech.', Bd. 44.
- Tahara, M. (1915).—"Cytological investigations on the root-tips of *Helianthus*" (Japanese), 'Bot. Mag. Tokyo', vol. 29.
- Tischler, G. (1922).—"Allgemeine Pflanzenkaryologie". Berlin.
- Tschenzoff, B. (1916).—"Die Kernteilung bei *Euglena viridis*", 'Arch. f. Protist', Bd. 36.
- de T'Serclaes, J. (1922).—"Le noyau et la division nucléaire dans le *Cladophora glomerata*", 'La Cellule', tom. 32.
- Van Camp, G. M. (1924).—"Le rôle du nucléole dans la caryocinèse somatique", 'La Cellule', tom. 34.
- Wager, H. (1904).—"The nucleolus and nuclear division in the root-apex of *Phaseolus*", 'Ann. Bot.', vol. 18.
- Zacharias, E. (1902).—"Ueber die 'achromatischen' Bestandtheile des Zellkerns", 'Ber. d. d. Bot. Ges.', Bd. 20.
- Zimmermann, A. (1893).—"Beiträge zur Morphologie und Physiologie der Pflanzenzelle", Bd. 2, 'Ueber das Verhalten der Nucleolen während der Karyokinese'. Tübingen.

EXPLANATION OF PLATE 11.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 1,675 diameters, and subsequently completed free hand. In

reproducing, the figures have been reduced to an enlargement of approximately 1,250 diameters. All of the figures are from the outer layers of the plerome and are printed with that side of the cell at the top which was originally directed toward the seed (except in the case of cross-sections).

Figs. 3, 4, 5, 13, 15, 16, 17, and 19 are from the Connecticut Field, and figs. 1, 6, 7, 9, 11, 12, 14, and 18 from the Winter Luxury variety of *Cucurbita pepo*; figs. 2 and 10 are from the Hubbard squash (*Cucurbita maxima*). The original preparations in case of figs. 5, 8, 13, 15, and 16 were from material fixed in Mottier's modification of Benda, stained according to the usual Benda method with alizarin crystal violet, and show the plastidome elements in the cytoplasm; figs. 6, 7, 9, 12, 14, and 18 are from root-tips fixed in vom Rath's fluid and stained with Fe-haematoxylin and eosin; figs. 1 and 11 are from preparations fixed in Bouin and stained with Fe-haematoxylin eosin; fig. 2, after fixation in Hermann and staining with Fe-haematoxylin; figs. 3 and 4, after fixation in Hermann and staining with safranin and light green; fig. 10 after fixation in strong Flemming and staining with Fe-haematoxylin; figs. 17 and 19 are from material fixed in corrosive sublimate and stained with safranin and light green.

PLATE 11.

Fig. 1.—Typical resting cell showing the large nucleolus.

Fig. 2.—Prophase.

Fig. 3.—Late prophase, with the nuclear membrane still intact.

Fig. 4.—Final prophase. The nuclear membrane has disappeared and the chromosomes are forming the metaphase plate.

Fig. 5.—Metaphase showing the nucleolus drawn out in the longitudinal axis of the spindle.

Fig. 6.—Metaphase. Nucleolus is dumb-bell-shaped with a narrow constriction in the equatorial plate. The two lobes of the nucleolus are not always so nearly equal.

Fig. 7.—Metaphase.

Fig. 8.—Metaphase showing unequal distribution of the nucleolus.

Fig. 9.—Metaphase. This example shows a very unequal distribution of the nucleolus. When the nucleolus is divided one large piece will go towards one pole, and a very small portion towards the other.

Fig. 10.—Cross-section through the metaphase plate showing the nucleolus within the plate of chromosomes. By focusing carefully it is possible to follow the nucleolus in different planes. In the plane of the chromosomes it is very small in diameter; at a higher or lower plane, the nucleolus is much larger; the smaller region obviously corresponds to the isthmus connecting the two larger portions in a dumb-bell-like figure such as is shown in fig. 6. When the Auerbach stain is used, the nucleolus is stained a brilliant red in contrast to the bright green of the chromosomes. In the Fe-haematoxylin stain the nucleolus takes an intense black and shows up clearly within the more lightly stained chromosomes of the metaphase plate.

Fig. 11.—Cross-section through a metaphase plate.

Fig. 12.—Metaphase showing a divided nucleolus, the two halves migrating to opposite poles.

Fig. 13.—Metaphase showing the two parts of the nucleolus still retaining evidence of the constriction.

Fig. 14.—A later stage than the two preceding figures, showing a metaphase with the nuclear fragments near or at the spindle poles.

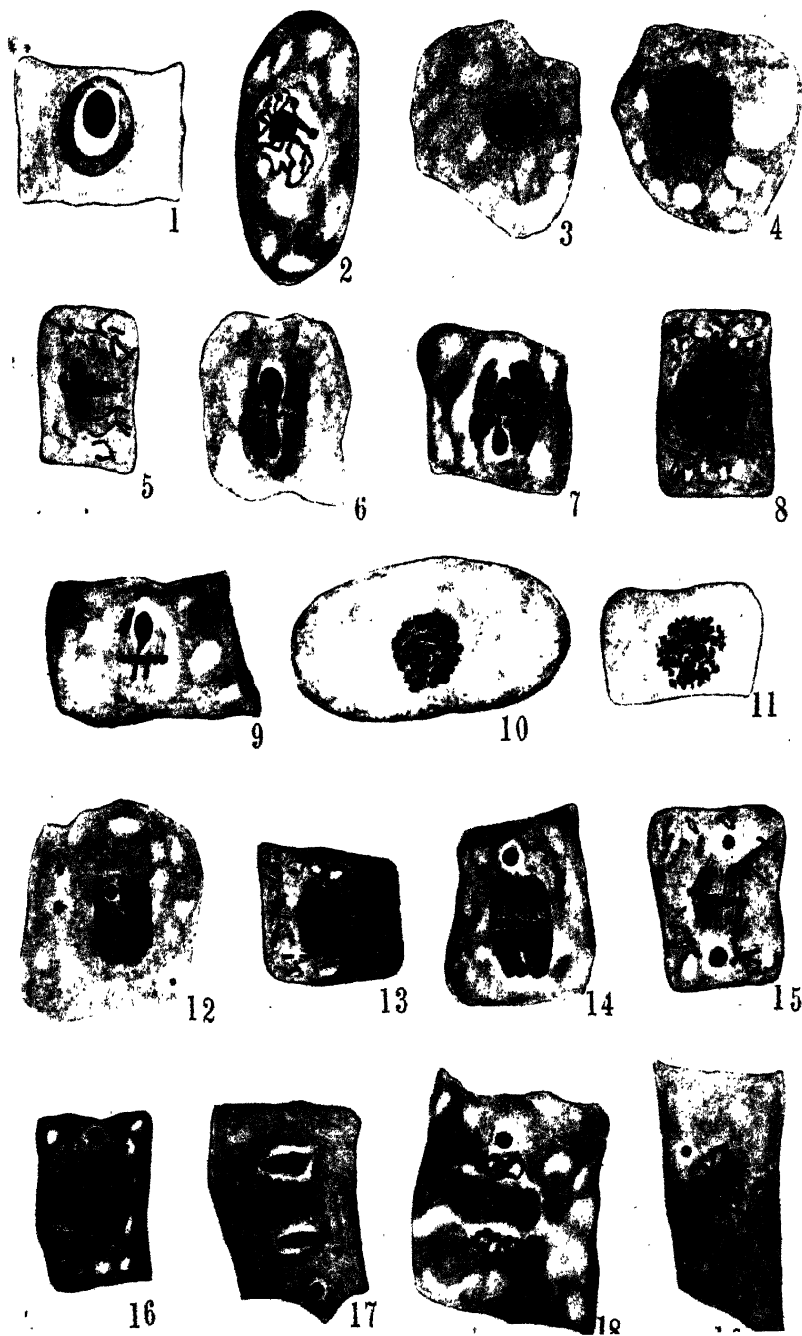
Fig. 15.—Metaphase showing the nuclear fragments at the spindle poles. It is to be noted that one fragment is considerably larger than the other.

Fig. 16.—Metaphase with approximately equal nucleolar fragments at the spindle poles.

Fig. 17.—Anaphase showing one nucleolar fragment in the cytoplasm. It is quite probable that the other nucleolar fragment was much smaller and has disintegrated.

Fig. 18.—Telophase showing one nucleolar fragment in the cytoplasm.

Fig. 19.—Telophase with one nucleolar fragment in the cytoplasm and clearly separated from the chromosomes. What is perhaps a new nucleolus can be made out forming within the chromosomes of the opposite daughter group.





The Conjugation of a Triploid Chilodon.

By

Mary Stuart MacDougall,

Agnes Scott College and The Marine Biological Laboratory.

With 16 Text-figures.

THE finding of a triploid *Chilodon* in the cultures of *Chilodon uncinatus* which had been exposed to the action of ultra-violet rays has been previously reported, MacDougall (1929). The present paper deals with the conjugation of this organism.

Chilodon uncinatus has been described many times. A brief description is included here for comparison only. It is a holotrichous ciliate, belonging to the family Chlamydodontidae. The ventral surface is very flat, and the dorsal surface curved, Text-figs. 1 and 2. The arrangement of the cilia, which are confined to the ventral surface, is characteristic. At the anterior end, on the left side, there is a zone along which a band of stronger cilia lead from a lateral angle to the mouth. From this zone, five rows of cilia take their origin, pass around the anterior end, and down the right side, the outermost row extending only about half-way down, the others ending at different points near the posterior end. On the left-hand side (right in the figure) four rows take their origin at the zone before mentioned. Two of these are short, extending about one-fourth the length of the body, and two extend to the posterior region. Two additional rows originate about one-fourth of the way from the posterior margin, and extend caudad.

The macronucleus is in the posterior region, is granular, and contains an endosome. This endosome contains a kinetic element designated by Calkins (1919) as an endobasal body.

The micronucleus is very close to the macronucleus, and is posterior to it. It also contains an endobasal body.

The oral basket, or 'Reusenapparat' is in the anterior region. Typically it contains ten trichites. The identity of the trichites is lost about half-way its length, where they seem to fuse, forming a tube. The basket is characteristically drawn out into a filament curved like a 'twice wound horn'.

TEXT-FIGS. 1 AND 2.

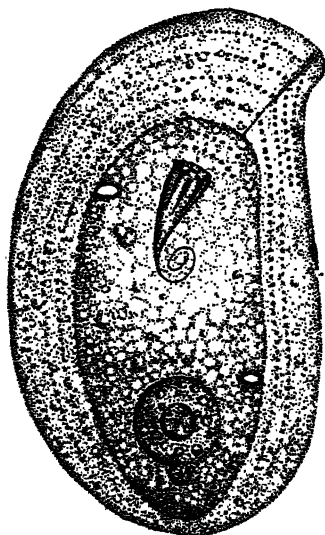
Fig. 1.—*Chilodon uncinatus*,
ventral view.

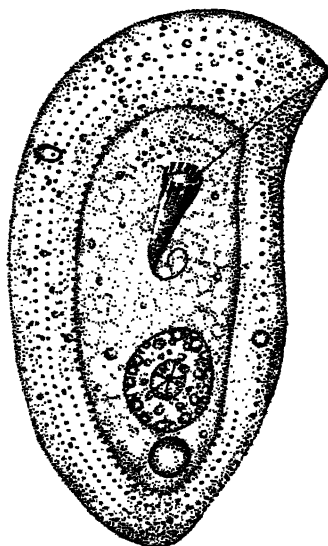
Fig. 2—Side view.

The triploid *Chilodon*, Text-fig. 3, differs from *Chilodon uncinatus* chiefly in the posterior portion. The ciliated margin in this region is wider, and the rows of cilia encircle it completely. The nuclei are nearer the centre of the body, and there seems to be twelve trichites in the pharyngeal basket. Although *Chilodon uncinatus* usually has ten trichites in its oral apparatus, the writer has seen individuals with twelve.

Details of the conjugation of *Chilodon uncinatus* have been described by Enriques (1908) and MacDougall (1925). The behaviour of the pharyngeal basket during conjugation has also been fully described.

A comparison of the details of the conjugation of the triploid organism with the diploid and tetraploid forms show very little difference. The behaviour of the odd number of chromosomes,

TEXT-FIG. 3.

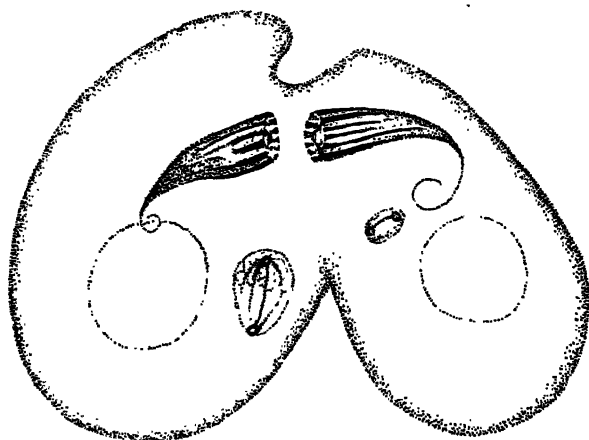


The Triploid Chilodon.

however, seems interesting enough to warrant the present investigation.

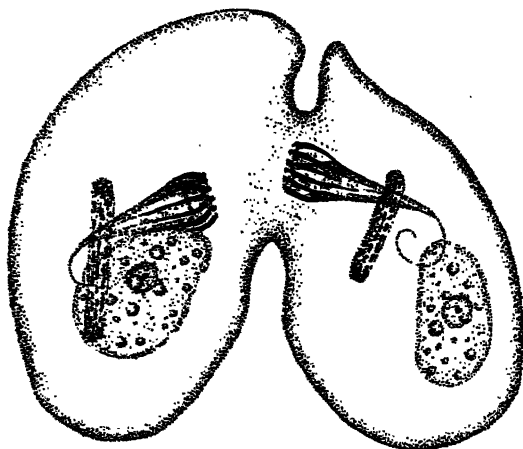
First Maturation Division.—In the early stage of maturation the endobasal body in the micronucleus divides, then a spireme in the form of a 'parachute' (Calkins, 1919) appears, Text-fig. 4. The spireme breaks up into strings of granules, Text-fig. 5, which later condense into six chromosomes, Text-fig. 6. The metaphase follows, the chromosomes having split, Text-fig. 7, six to go to each pole of the spindle. A pairing of the chromosomes, preparatory to the resting stage, has been observed in *Chilodon uncinatus* in the diploid and tetraploid forms. This phenomenon was also observed in the triploid form, Text-fig. 8.

TEXT-FIG. 4.



The 'Parachute Stage'.

TEXT-FIG. 5.



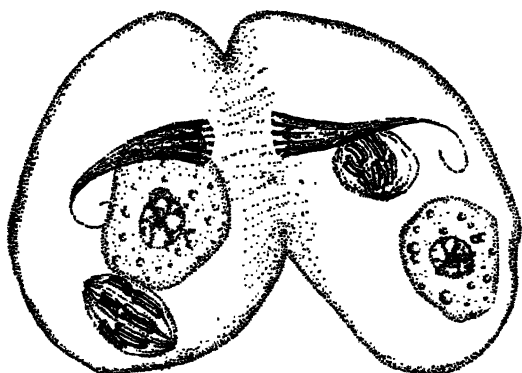
The micronucleus drawn out into strings of granules.

TEXT-FIG. 6.



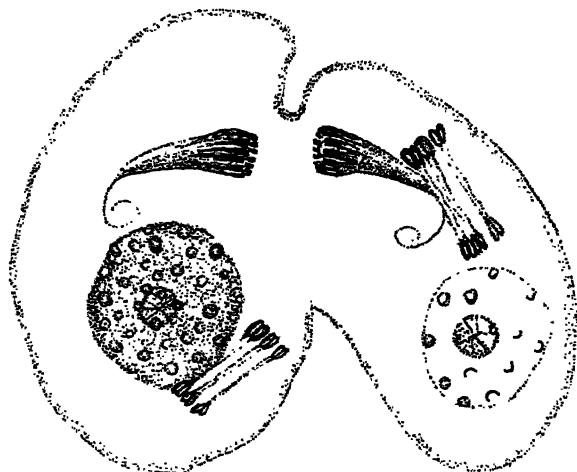
Six chromosomes on spindle.

TEXT-FIG. 7.



Metaphase.

TEXT-FIG. 8.



Pairing of the chromosomes preparatory to the resting stage.

The Second Maturation Division.—This is the reduction division. After telophase of the first maturation division, the daughter nuclei go into a resting stage. Sometimes both of these nuclei divide, and sometimes only one. The endobasal body divides, as in the first maturation division, and a

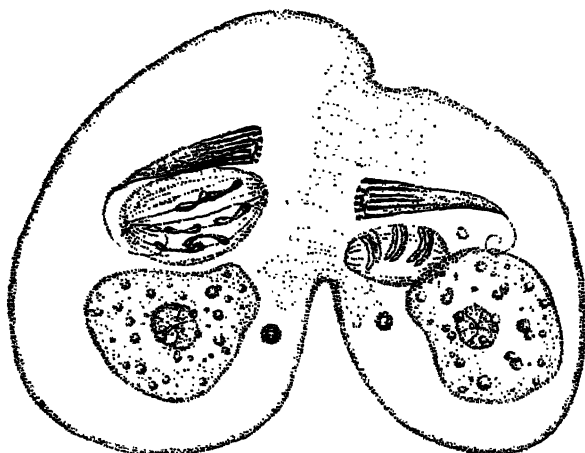
spireme is formed, Text-fig. 9. No granule stage was found in the second maturation division, probably due to the fact that the material was not very abundant. Six chromosomes appear, three of which go to each pole of the spindle, Text-fig. 10. The

TEXT-FIG. 9.



Beginning of the second maturation division. Spireme stage.

TEXT-FIG. 10.



The reduction division.

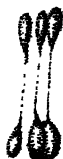
reduction division is thus accomplished. Preparatory to the resting stage, which follows, two chromosomes pair, and one remains unpaired, Text-fig. 11.

In the previous studies of the diploid and tetraploid forms of *Chilodon uncinatus*, the endobasal body could not be found after the spindle was formed. In the triploid organism, the endobasal body was found to be present at each end of the

spindle in all of the stages. Failure to find them in the other material was probably a matter of technique.

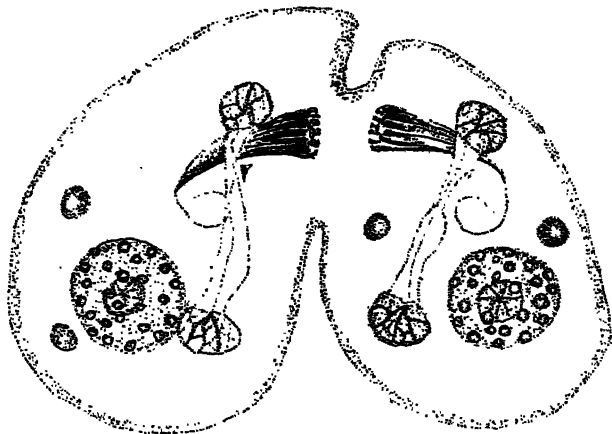
The Third Maturation Division.—Following the resting stage of the second maturation division, Text-fig. 12, the pronuclei move into the region of the protoplasmic bridge,

TEXT-FIG. 11.



Pairing of the chromosomes preparatory to the resting stage of the second maturation division.

TEXT-FIG. 12.

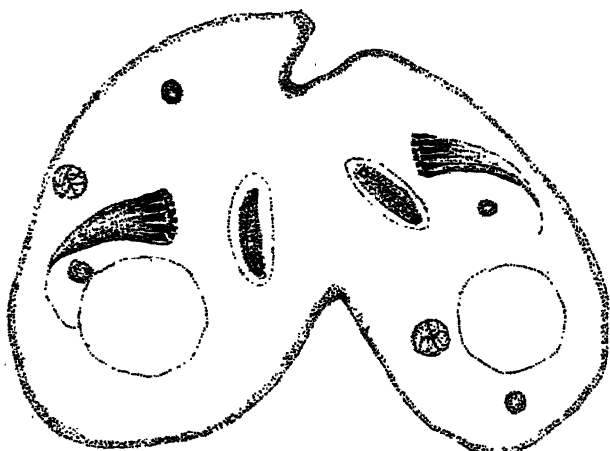


Resting stage of the second maturation division.

Text-fig. 13. As in the other maturation divisions, the endobasal body divides, and a spireme is formed. The spireme is very dense. Three short rows of granules appear, Text-fig. 14, and these condense into three chromosomes. The manner of division of these chromosomes has not been accurately determined. No clear figures have been found in the material which show a split in the chromosomes. After division of the chromosomes the

spindle pulls out, and three chromosomes are seen at each end, Text-fig. 15. The interchange of nuclei then takes place. It will be noted in both Text-figs. 15 and 16 that two of the chromosomes are paired, and one remains unpaired. Extended observations have yielded no light as to the significance of this

TEXT-FIG. 13.



Division of the endobasal body. Third maturation division.

TEXT-FIG. 14.



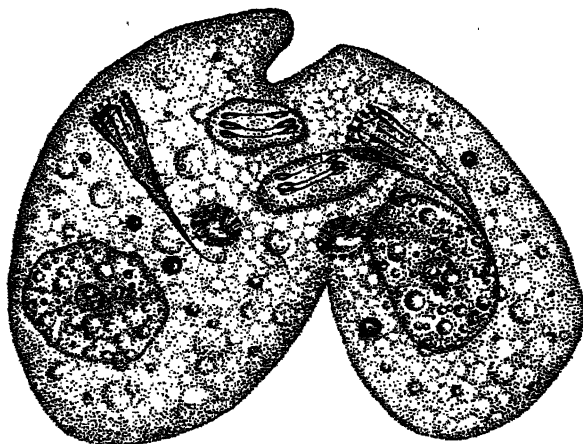
Granule stage. Third maturation division.

pairing preceding the resting stage. When the migrating and stationary nuclei touch, the membranes disappear at the point of contact, and the two sets of chromosomes come to lie side by side, Text-fig. 16. After fusion of the nuclei there is a resting stage, at which time the animals separate.

Only the important stages of conjugation have been included here. All the other details are identical with those of the diploid and tetraploid forms of *Chilodon uncinatus*.

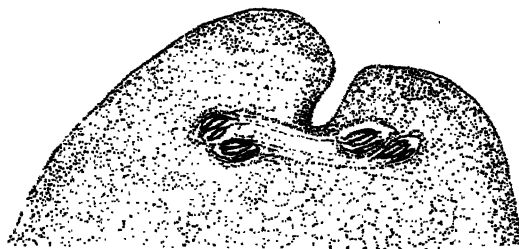
Reorganization of the exconjugant is exactly similar to that described for the forms referred to above.

TEXT-FIG. 15.



Interchange of Nuclei.

TEXT-FIG. 16.



Late stage of the interchange of nuclei.

LITERATURE CITED.

- Calkins, Gary N. (1926).—‘The Biology of the Protozoa’. New York, Lea and Febiger.
- Enriques, P. (1908).—“Die Conjugation und sexuelle Differenzierung der Infusorien”, ‘Archiv für Protistenkunde’, Bd. 12, pp. 213–74.
- MacDougall, Mary Stuart (1925).—“Cytological observations on Gymnostomatous ciliates, with a description of the maturation phenomena in diploid and tetraploid forms of *Chilodon uncinatus*”, ‘Quart. Journ. Micro. Sci.’, vol. 69, Part III.
- (1929).—“Modifications of *Chilodon uncinatus* produced by ultra-violet light”, ‘Journ. Exp. Zool.’, v. 54, no. 1.

The Early Prophases of the First Oocyte Division as seen in Life, in *Obelia geniculata*.

By

G. H. Faulkner, B.Sc. (Edin.),

Demonstrator in Zoology in Bedford College, University of London.

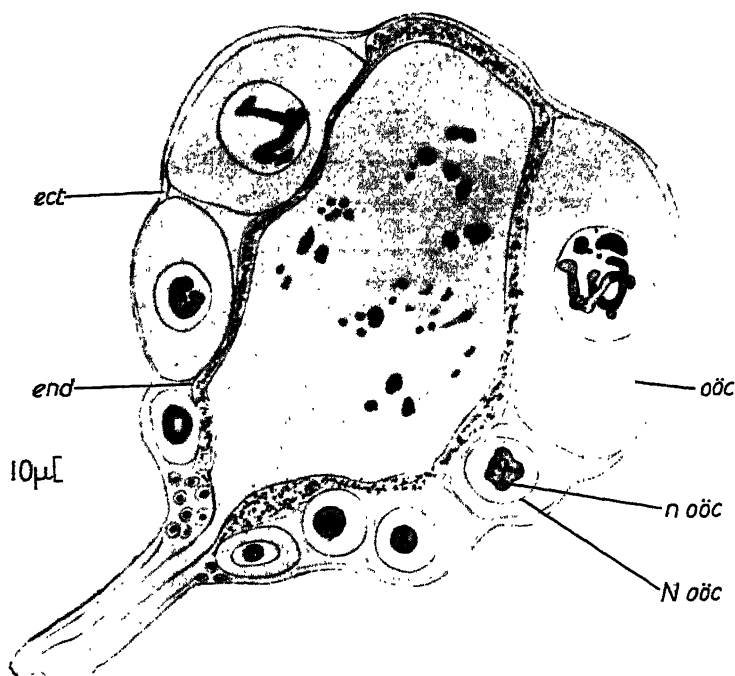
With 23 Text-figures.

Obelia is a very suitable subject for the observation of living oocytes, for although the eggs attain a comparatively large size, e.g. 0.2 mm., the yolk deposited in them is perfectly colourless and transparent, and allows the whole of the nuclear cycle to be examined in considerable detail; a $\frac{1}{8}$ -inch objective was used in this case, but an even higher power could be used equally well. As the medusa itself is perfectly transparent, the eggs may be examined in situ in the gonads, hence they undergo the minimum amount of handling and interference, and remain healthy during prolonged observations. It is even possible to keep isolated individuals alive and examine the gonads from day to day.¹

The following description of the oocyte prophases is based entirely on observations on the living egg, and all the figures (with the exception of the spermatocyte nucleus drawn in Text-fig. 23) were drawn from life with the aid of a Leitz-Zeichen-Okular, under a $\frac{1}{8}$ -inch objective. In most cases the medusae were examined within the first few hours after they were brought into the laboratory, but occasionally they were left in an aquarium during the night and examined the following morning.

During the sexual season, each gonad contains a group of

¹ This piece of work was performed in the Marine Biological Laboratory, Plymouth, during the months of July and August 1928; the author wishes to express her thanks for the use of the University of London table in the Laboratory at the time.

TEXT-FIG. 1¹.

A complete ovary, semi-diagrammatic.

LETTERING OF TEXT-FIGURES 1-23.

ch.biv., bivalent chromosomes; *ch.univ.*, univalent chromosomes; *ect.*, ectodermal epithelium; *end.*, endodermal epithelium of gastric pouch; *N.ooc.*, nucleus of oocyte; *n.ooc.*, nucleolus of oocyte; *N.sp.*, nucleus of spermatocyte; *sp.*, chromosomal spireme; *vac.*, vacuole.

oocytes at different stages of growth; small resting cells are present, and there are generally about six larger oocytes in which the nucleus has passed from the resting condition into

¹ All the figures, except Text-fig. 23, were drawn from life with the aid of a Leitz-Zeichen-Okular. Text-figs. 2 to 22 were all drawn at the same magnification, using a $\frac{1}{4}$ -inch objective; the larger eggs, however, were subjected to a certain variable amount of pressure under the coverslip, hence the measurements are not in all cases strictly comparable.

the active pre-maturation phases. Text-fig. 1 shows a typical ovary drawn from life semi-diagrammatically, as seen when

TEXT-FIGS. 2-12a.

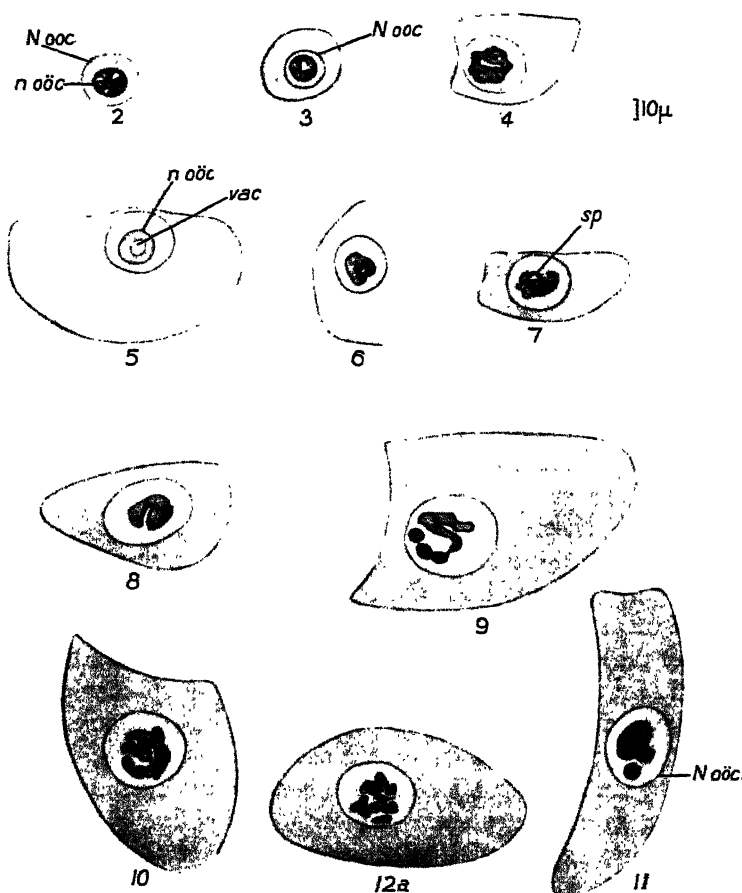


Fig. 2.—Nucleus of oocyte.

Figs. 3-12a.—Drawings of oocytes.

focused deeply below the surface. The oocytes are arranged in a single series round the exterior of an endodermal pouch: there is one such pouch developed on each of the four radial canals; the four lobes thus formed project from the sub-umbrellar

surface and hang freely into the concavity of the bell. Externally the oocytes are covered by a thin ectodermal cellular layer, hence they occupy a position between endo- and ecto-derm. The youngest oocytes are situated proximally at the base of the gonad and the oldest are the most distal; frequently, instead of the symmetrical arrangement seen in the figure, there is one large terminal oocyte.

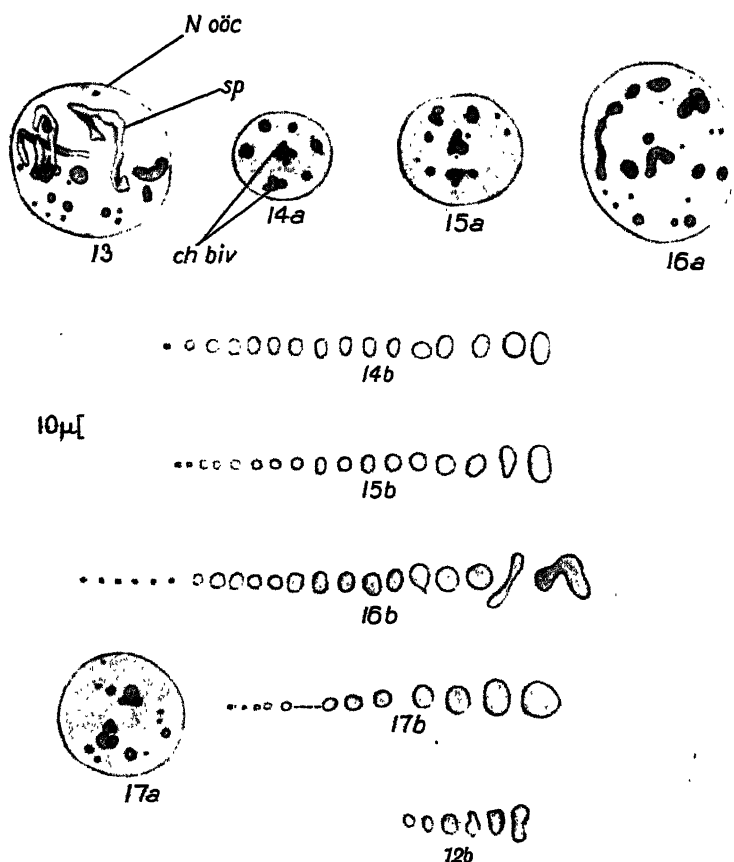
In the young resting oocytes at the base of the ovary the nucleus is almost entirely filled by the nucleolus (Text-figs. 1 and 3). This nucleolus is refringent in appearance, and though it is almost colourless, appears to have a slight greenish-grey tint against the perfectly colourless surroundings. It is more or less vacuolated; there may be one large vacuole or many small ones, and the pattern of these changes under observation (Text-figs. 2-5).

As the cell increases in size the nucleus enlarges also, but the size of the nucleolus remains practically unchanged, its diameter being approximately 15μ . When the cell reaches a diameter of $50-75\mu$ (nucleus being *c.* 30μ) differentiation of the nucleus begins and a series of typical oocyte prophases follows. The chromosomes become defined as such, and later disappear again, leaving the nucleus clear.

The first stage in the differentiation of the nucleus is the elongation of the spherical nucleolus: the details of this process vary in different cases, but the final result is the transformation of the nucleolus into an elongated ribbon, which by virtue of its subsequent behaviour must be considered to be a spireme. Text-figs. 6, 7, and 8 show three oocytes in which the nucleolus is in the process of elongation. Text-fig. 6 shows the type most frequently seen, in which at the very first stage the nucleolus assumes a C-shape; Text-fig. 8 shows a slight modification of this type of nucleolus. Text-fig. 7 shows a cell whose nucleus is at approximately the same age, but in which the nucleolus is producing a long slender ribbon instead of a short thick one. This kind of ribbon seems to be derived from the nucleolus by a kind of spreading process which suggests the exact reverse of what would happen if a coiled length of some plasticene-like

ribbon were taken in the hands and compressed into a smooth ball. The ribbons derived from nucleoli such as are figured in

TEXT-FIGS. 12b-17b.



Oocyte nuclei, and chromosomes.

Text-figs. 6 and 8 elongate more or less subsequently, becoming correspondingly slender at the same time.

During the next phase the spireme breaks up by a series of transverse fissions, and finally, in place of the continuous ribbon, there are spherical fragments of various sizes scattered through

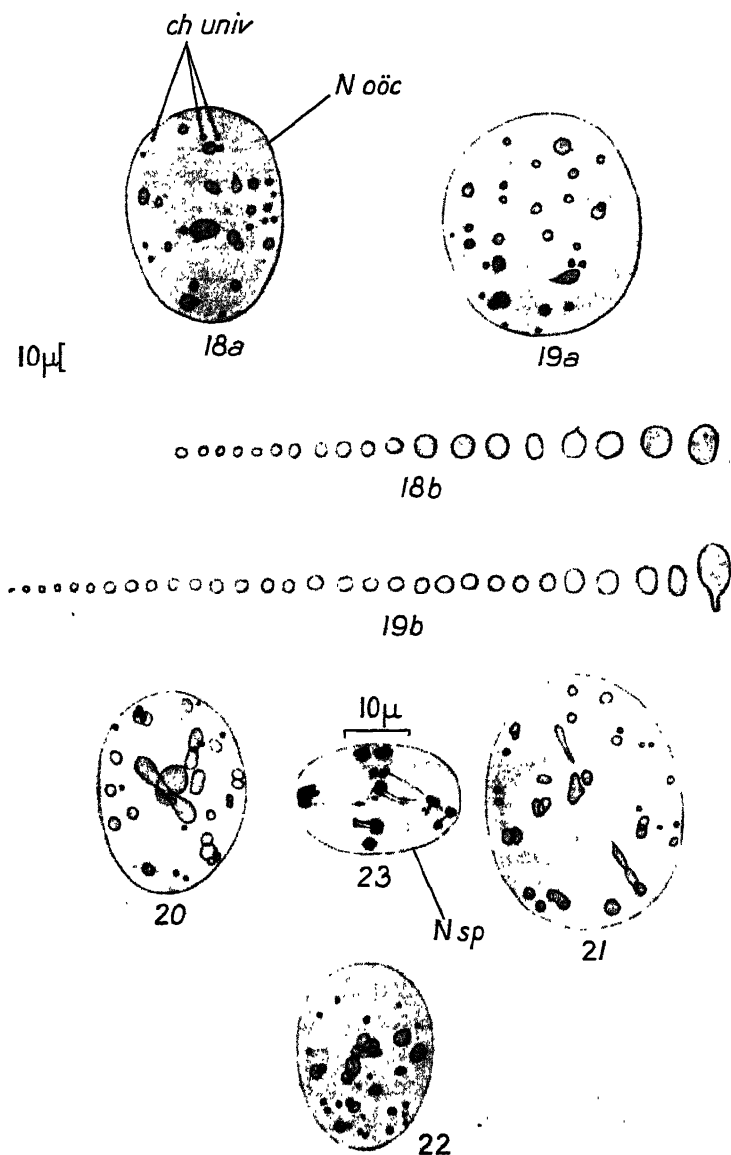
the nucleus. The details of the process of fragmentation vary considerably. Text-figs. 9 to 13 illustrate nuclei in which the breaking up of the spireme is in process. In Text-fig. 9 the nucleolus is still elongated and is segmenting regularly at one end; such a nucleus might be a later stage of a nucleus such as that in Text-fig. 6 or in Text-fig. 8. Text-fig. 11 shows an almost unique case in which the nucleolus began to fragment without undergoing any initial elongation. Text-fig. 10 shows a later stage of the Text-fig. 7 type of spireme: the coiled knot-like formation is now less compressed, and the coil has segmented into sections—the divisions occurring simultaneously throughout the entire length of the ribbon.

Text-fig. 13 differs from the previous four in that the spireme has become more elongated, also in the fact that the divisions are not all happening synchronously; hence several long pieces of spireme are coexistent with numbers of small fragments. Such irregular nuclei are very common.

The completion of the fragmentation phase leaves the nuclei in the condition seen in Text-figs. 12, 14, 15, 16, and 17. The spireme has entirely broken up into more or less spherical fragments, and it is concluded that each fragment is a pair of homologous chromosomes. The evidence for calling them chromosomal lies in two facts. Firstly, when ideal cases of the phase are found (i.e. cases in which the divisions synchronize accurately), the number of these fragments is constant; further, the fragments are not of equal size but show a constant pattern of heteromorphism. It is assumed that these two features are actually characteristic of chromosomes.

In Text-figs. 14*a* to 17*a* the chromosomes are sketched in the nuclei to show their arrangement, but are actually rather smaller than they should be. They were drawn so to add clearness to the drawings; in Text-figs. 14*b* to 17*b* the same chromosomes are drawn with the aid of a camera lucida and arranged in graduated series for comparison. There is in each case one element larger than the rest, and there are two minute fragments; between these two extremes there is a fairly even gradation. Two or three of the fragments are always conspicu-

TEXT-FIGS. 18-23.



Figs. 18-22.—Oocyte nuclei, and chromosomes. nucleus in section.

Fig. 23.—Spermatocyte

ously larger than the rest. The number of chromosomes counted at this phase is seventeen, and as the elements are later shown to be bivalent, this is equivalent to the haploid number.

Text-fig. 12*a* is a rather special case of a nucleus at this particular phase. It is unusual to find such an early breaking of the spireme as this; the divisions must have occurred at the beginning of unravelling of a spireme of a type intermediate in form between those of Text-figs. 6 and 7. In Text-fig. 12 indication of fifteen blocks could be found, hence it is an almost typical example of a completely segmented spireme.

In the following stages of growth each of these bivalent chromosomes divides into two equal halves. At first the two halves resulting from the division remain adjacent, but later they separate so that finally fragments are evenly distributed over the nucleus. Text-figs. 18 to 22 show such nuclei: various degrees of pairing and of dispersion are seen, and, as is expected, heteromorphism parallel to that of the previous phase is conspicuous. Text-figs. 18*b* and 19*b* are camera lucida tracings of the chromosomes of the corresponding nuclei. Text-fig. 18 has thirty-four chromosomes countable, but only the larger ones were accurately traced. In Text-fig. 19 only thirty chromosomes are seen and these are all traced. In Text-fig. 20 the larger fragments were also traced: in this case there are thirty-one small, presumably univalent elements, and two larger ones which are probably still bivalent but showing signs of fission. Hence the number is estimated at thirty-five here. In Text-figs. 21 and 22 there are thirty-four chromosomes present in each.

It is therefore concluded that seventeen and thirty-four are the n and $2n$ (i. e. haploid and diploid) numbers respectively for the chromosomes of *Obelia*.

In connexion with this breaking up of the spireme into bivalent elements, it is of interest to note that Hargitt (1916) mentions that in *Clava leptostyla* he fails to find a syndesis phase, and suggests that it may be that the chromatin 'condenses into half the usual number of bodies', since the chromosomes appear on the first maturation spindle in the reduced

number. Another comparable case is given by Gates, as mentioned in the historical section, p. 239.

One other point of theoretical importance shown by these chromosomes is the indication that the large chromosome seen in the bivalent group represents an unequal (XY) pair. In the univalent condition the largest chromosome is often irregular in form, most commonly hammer-shaped or wedge-shaped; it generally lies in the centre of the nucleus. In the nucleus, represented in Text-fig. 21, it is believed that the large chromosome had divided immediately before inspection and that the two chromosomes in the centre of the field, one larger and triangular and the other smaller and spherical, resulted from the division; these two chromosomes were drawn as accurately as possible with the camera lucida, but the rest of the elements were sketched free-hand. It should be emphasized here that the paired arrangement of the individual chromosomes for some time after their separation is much more conspicuous and convincing in real life than it is in the figures; this is necessarily so, for the chromosomes are in reality distributed through the interior of a spherical chamber, and hence are separated from each other by distances of depth as well as the lateral distances which alone can be represented on a plane surface. The appearance of the nucleus from which Text-fig. 21 was drawn was such that it left no doubt that the two chromosomes under discussion were really members of a pair, although no further proof can be produced. Probably the comma-shaped form in Text-fig. 20 represents the corresponding XY pair in this nucleus, since as mentioned above there is indication that this is a bivalent element.

After the chromosomes have become defined in this manner, they disappear from view again and leave the nuclear vesicle quite clear. The disappearance is due to the further fragmentation—or disintegration—of each chromosome into a group of small globules. The fragmentation may occur gradually, in which case the fragments become steadily smaller and at the same time more numerous; on the other hand, each chromosome may resolve itself at one moment into a group of minute

globules which at first remain as a group, but later become dispersed. As a result of this process the chromosomes lose their discrete visible individuality entirely. Wilson ('The Cell', 1927) discusses the apparent fading of the chromosomes at this stage of oogenesis and mentions the fact that several authors describe this particular kind of temporary dispersion of the chromosomes. He expresses the opinion that the chromosomes 'do not disappear by breaking up into a structureless magma or mass of fine granules, as some observers have concluded; there is reason to believe that this account rests on faulty technique'. In the present case there can be no question of imperfect technique, and I feel convinced from personal observation of these nuclei that the condition is normal and not pathological.

The series of stages described and figured here are not always achieved in the orderly and ideal sequence in which they have been described. The phases often overlap each other to a considerable extent, so that nuclei are constantly found in which elongated sections of spireme are present together with numbers of bivalent, or even univalent, chromosomes. Further, the stage characterized by the presence of the bivalent elements may be masked by the precocious division of a few of these elements, so that a nucleus such as that drawn in Text-fig. 16 results: in this there are four fragments in excess of the normal seventeen; these may have been derived from division of the smaller bivalents; sometimes they seem to arise by separation from one of the larger chromosomes. Then similarly the typical 'chromosomal' phase in which the thirty-four chromosomes are displayed, may be omitted as an actual fact, and counting rendered impossible, owing to the early and irregular fragmentation of some individuals. However, a sufficient number of typical stages has been found to demonstrate repeatedly the actual course of events.

Whether the final division of the bivalent chromosomes represents a further transverse, or a longitudinal division, is not known. No trace of a longitudinal fission was ever seen in the spireme or in any portion of it; in view of the fact that irregularities are met with so frequently in all stages of the cycle of events,

and that there is certainly an overlapping of the univalent condition of some pairs of individuals and the spireme condition of others, it might be expected that if the final division were longitudinal, some trace of the split might have been indicated occasionally as an anachronism. As this never occurred, it is concluded that the chromosomes are probably arranged in a single linear series on the spireme, members of homologous pairs being adjacent.

There was one other feature that was sometimes noticed in these oocyte nuclei. On several occasions the nuclei contained long needle-shaped crystals, though they appeared in every way perfectly healthy and otherwise normal. The crystals were long and slender, being equal in length to the diameter of the nucleus. They were colourless, and varied in number from one single crystal to a star-shaped group composed of about two dozen.

NOTES ON THE APPEARANCE OF THE OOCYTES IN FIXED AND STAINED PREPARATIONS.

The cytological details of these nuclei as seen in fixed preparations will not be discussed in the present paper. However, there are a few points which more particularly concern the chromosomes and which should therefore be mentioned.

Medusae were fixed and mounted, either as whole mounts (stained and unstained) or as sections. Fixation was with Flemming-without-acetic, Bouin, and Allen's chromic-urea-Bouin. Subsequent staining was with either iron haematoxylin, iron haematoxylin and eosin, or (after F.w.a.) safranin and light-green. These sections have confirmed every step of the above account.

Previous authors have described these oocytes as seen in sections, as mentioned in the historical paragraphs below. Jorgensen (1913) in particular has given an account with a complete set of figures. He claims to see faintly staining oxyphilic chromosomes in the larger nuclei, structures entirely independent of the nucleolus. I have repeated the precise method used most successfully by Jorgensen (namely, F.w.a., and safranin and light-green), but I have failed to find them. In

some preparations, especially the Bouin-fixed, appearances similar to those figured by Jorgensen are seen, but it is assumed that they are artefacts; in the most perfectly fixed preparations (i. e. those fixed with warm F.w.a.) the nucleoplasm is uniformly granular and perfectly homogeneous, the only visible structures inside the nuclear membrane being the nucleolar fragments.

One other method was tried to test the staining properties of these nucleolar fragments, namely, Feulgen's 'Nuklealreaktion'. Although the method was modified in various ways, it was found impossible to stain any structure in these oocytes by the reagent. This negative evidence, however, is not considered to have very much significance. Previous authors have found that it is not to be regarded as an absolute criterion for diagnosing chromosomes. Bělař (1926) says that it is doubtful whether the test is specific for all nuclear phases, but that it is useful for resting stages. Harvey (1929) also finds that in the oocytes of *Carcinus maenas*, the chromosomes do not show the reaction after the bouquet stage; the corresponding stage in these *Obelia* oocytes occurs before the commencement of the growing stages described here, hence it is not unique that the chromosomes should fail to give the reaction in the dispersion phase.

An attempt was made to corroborate the above conclusions by making a count of the chromosomes in the spermatocyte. It has not been possible to do this with certainty, but it is evident that the number of chromosomes countable in the spermatocyte is in the neighbourhood of fifteen pairs. Text-fig. 23 was drawn from a spermatocyte nucleus in which it was possible to make an approximate count of the chromosomes. This helps to confirm the conclusion reached previously that the nucleolar fragments really do represent chromosomes.

HISTORICAL.

The nuclei of the oocytes of *Obelia* have been mentioned and figured by several previous authors. The earliest account is that of Merejkovsky (1880 and 1883), who apparently observed them in life and described the various phases in the division of

the nucleolus. Metschnikoff (1886) also observed the fragmentation. Both authors, however, missed the fact that the fragments had a recognizable individuality in all the different nuclei. Merejkovsky mentions that at one stage there are 'plusieurs dizaines' fragments countable in the nucleus, referring no doubt to the thirty-four chromosomal elements; the author obviously regards this particular stage as noteworthy and of the nature of a landmark, and must have realized that the divisions were not entirely indiscriminate and steadily continuous.

Trinci (1906) describes *Obelia* oocytes, but his observations were made on serial sections, and he simply states that the nucleolus fragments, without discussing the significance of this. He considers that the chromosomes exist in an unstainable condition during the growth stages. It is of great interest to notice that in describing these nuclei Trinci states that by the division of the nucleolus there result 'piu decine di corpore rotundi'. These, presumably the bivalent chromosomes, divide further and give more than a hundred fragments, then are entirely dispersed. In another place he states that 'nelle uove piu grandi se ne incontrano talora anche una trentina'. Hence he also recognizes the occurrence of a chromosomal phase of approximately thirty, but does not attach any importance to it.

Jørgensen's more recent account has already been mentioned (p. 235); this actually adds nothing to Trinci's previous paper.

Comparable fragmentation of the nucleolus in other Coelenterates is described by many authors, in fact Trinci (1905) groups Coelenterate eggs into two classes, those in which the nucleolus remains single and those in which it subdivides. Some of these authors claim that there is a relationship between nucleoli and chromosomes, that the nucleolus is in fact more or less chromatinic.

Stschelkanowzew (1906) describes the origin of the nucleolus in *Cunina* from the chromosomes.

G. T. Hargitt (1909) states that the nucleolus of *Pennaria* may contain a little chromatin. C. W. and G. T. Hargitt (1910) mention that they have seen an appearance suggesting that nucleolar material was passing into the chromosomes, but add

that the appearance may not be significant. G. T. Hargitt (1918) states that the nucleolus of *Campanularia* arises at least in part from the chromatin.

Schaxel (1910 i and ii) states that in *Pelagia noctiluca* the chromosomes condense to form the nucleolus; the same author in 1911 adds two more examples of the same condition, namely, *Forskalia* and *Agalma* (*Halistamma*).

Hickson (1893) describes fragmentation of the nucleolus in *Distichopora*, but does not discuss the nature of the fragments.

Among the large mass of literature dealing with the subject of nucleoli, the following authors admit that nucleoli may be more or less chromatinic:

Flemming, 1882; R. Hertwig, 1884, 1896, and 1898; O. Hertwig, 1900; Carnoy; Macallum (basing his conclusions on specific chemical chromatin tests), 1895; Went, 1897; Hartmann, 1902; Gunther, 1904; Jannsens and Willems, 1908; Jordan, 1910; Lundegardh, 1912; Tamura, 1923.

Other examples of nucleoli showing precisely the same relationship to chromosomes as in *Obelia* (i.e. a nucleolus consisting of the massed chromosomes) are given by the following authors: Blochmann, 1882, in *Neritina fluviatilis*; Moll, 1893 (and subsequent authors), in *Spirogyra*; R. Hertwig, 1894 and 1898, in *Actinosphaerium*; Wilson, 1901 (and subsequent authors), in *Echinoderms*; Goldschmidt, 1902, in *Polystomum*; Katheriner, 1904, in *Gyrodactylus*; Browne, 1913, in *Notonecta*; and Ashworth, 1923, in *Rhinosporidium seeberi*.

The reason that Trinci and Jörgensen missed the essential chromosomal nature of the fragments was probably that they examined only sectioned gonads. The features which first suggest chromosomes, namely, their constant number, constant heteromorphism, and the paired arrangement at one time, are all masked in sections, as the nuclei are so large in fully developed oocytes that they extend through about ten sections of normal thickness (e.g. 5μ).

The particular type of short, thick spireme that is seen in

Obelia is perhaps less common than the elongated coiled type. A somewhat similar spireme is described by Stout in *Carex*, and another by Gates in *Oenothera*, as mentioned on p. 233.

The last point of interest arising from these observations concerns the visibility of the chromosomes in life: nucleoli are generally visible but chromosomes have only seldom been described. Schneider, as early as 1873, found that chromosomes could be demonstrated in fresh *Platyhelminth* eggs by treating them with dilute acetic acid.

The previous account which most nearly resembles the case of *Obelia* is that by Mulsow (1911 and 1912) of the spermatogenesis of *Ancyracanthus*. The author confirms all his observations on living material, and he figures the ripe sperms in which the chromosomes are plainly visible as small refractive spherical granules, very reminiscent of those of *Obelia*.

Tischler (1910) has described chromosomes in living banana pollen cells.

Bělař (1927 and 1928) has made observations on the maturation divisions of the spermatocytes of *Chorthippus* and has followed the chromosome cycle in the living cell, comparing individual cells in the living and fixed conditions, by means of micro-photographs.

Chromosomes in living cells have also been described by Chambers; sometimes they are said to come into view when the cell is injured by introducing a micro-dissecting needle, but in other cases (Chambers and Sands, 1923) are visible in the normal untouched cell. Also they have been seen in dividing cells in tissue cultures, as described and figured by Strangeways (1924).

SUMMARY.

1. The nucleolus of the resting oocyte represents a condensed chromatic spireme, hence it is identical with the total chromosomal contents of the nucleus.
2. During the early growth phases of the oocytes, the nucleolus elongates and fragments. Each fragment has been identified as a pair of homologous chromosomes indistinguishably united;

later each of these bivalent elements divides in half, and the individual chromosomes are thus separated. The two components of the largest bivalent element are unequal in size, and probably represent an XY pair.

3. The chromosomes can be counted either at the bivalent or at the univalent phase, the numbers obtained being seventeen and thirty-four respectively.

4. At a still later stage the chromosomes fragment into numerous small globules, which become evenly distributed over the nucleus.

5. The whole of the account is based on observations made on living oocytes.

BIBLIOGRAPHY.

Ashworth, J. H. (1923).—"On *Rhinosporidium seeberi* (Wernicke, 1903), with special reference to its sporulation and affinities", 'Trans. Roy. Soc. Edin.', liii.

Bělař, K. (1926).—"Die Formwechsel der Protistenkerne", 'Erg. und Fortschr. der Zool.', vi.

— (1927).—"Beiträge zur Kenntnis des Mechanismus der indirekten Kernteilung", 'Naturwiss. Berlin', 15, no. 36.

— (1928).—"Über die Naturtreue des fixierten Präparats", 'Zeit. für Abstamm. und Vererb.', Suppl. 1.

Blochmann, F. (1882).—"Über die Entwicklung der *Neritina fluviatilis* Mull.", 'Zeit. wiss. Zool.', 36.

Browne, E. N. (1913).—"A study of the male germ cells in *Notonecta*", 'Journ. Exp. Zool.', xiv.

Carnoy (1884).—"La Biologie Cellulaire", 'Lierre'.

Chambers, R., and Sands, H. C. (1923).—"A dissection of the chromosomes of the pollen mother cells of *Tradescantia virginica* L.", 'Journ. Gen. Physiol.', 5.

Flemming, W. (1883).—"Zellsubstanz, Kern und Zelltheilung". Leipzig.

Gates, R. R. (1908).—"A study of reduction in *Oenothera rubrinervis*", 'Bot. Gaz.', xlv.

Goldschmidt, R. (1902).—"Untersuchungen über die Eireifung, Befruchtung und Zelltheilung bei *Polystomum integerrimum* Rud.", 'Zeit. wiss. Zool.', lxxi.

Gunther, K. (1904).—"Ueber den Nukleolus im reifenden Echinodermei und seine Bedeutung", 'Zool. Jahrb. Anat.', xix.

Hargitt, C. W. (1911).—"Some problems of Coelenterate ontogeny", 'Journ. Morph.', 22.

- Hargitt, C. W. and G. T. (1910).—"Studies on the Development of Scyphomedusae", *ibid.*, 21.
- Hargitt, G. T. (1909).—"Maturation, Fertilization, and Segmentation of *Pennaria tiarella* (Ayres) and of *Tubularia crocea* (Ag)", 'Bull. Mus. Comp. Zool. Harvard', 53.
- (1913).—"Germ Cells of Coelenterates. I. *Campanularia flexuosa*", 'Journ. Morph.', 24.
- (1916).—"Germ Cells of Coelenterates. II. *Clava leptostyla*", *ibid.*, 27.
- Hartmann, M. (1902).—"Ovarialei und Eireifung von *Asteria glacialis*", 'Zool. Jahrb. Anat.', xv.
- Harvey, L. A. (1929).—"The oogenesis of *Carcinus maenas* Penn., with special reference to yolk-formation", 'Trans. Roy. Soc. Edin.', lvi.
- Hertwig, O. (1909).—"Allgemeine Biologie", 3e Auflage.
- Hertwig, R. (1884).—"Über die Kerntheilung bei *Actinosphaerium*", 'Jena. Zeitsch. f. Naturwiss'.
- (1896).—"Über die Entwicklung des unbefruchteten Seeigelleies", 'Fest. Geburtst. Gegenbaur'.
- (1898).—"Über Kerntheilung, Richtungskörperbildung und Befruchtung von *Actinosphaerium eichorni*", 'Abh. Kgl. bayer. Akad. Wiss.', 2Kl., xix.
- Hickson, S. J. (1894).—"The early stages in the development of *Distichopora violacea*, with a short essay on the fragmentation of the nucleus", 'Quart. Journ. Micr. Sci.', 35.
- Jannsens and Willems (1903).—"La spermatogenèse dans l'*Alytes obstetricans*", 'La Cellule', xxv.
- Jordan, H. E. (1910).—"A cytological study of the egg of *Cummingia*, with special reference to the history of the chromosomes and the centrosome", 'Arch. Zellf.', 4.
- Jørgensen, M. (1913).—"Morphologische Beiträge zum Problem des Eiwachstums", *ibid.*, 10.
- Katherineer, L. (1904).—"Ueber die Entwicklung von *Gyrodactylus elegans* v. Nrdm.", 'Zool. Jahrb.', Suppl. VII.
- Lundergårdh, H. (1912).—"Chromosomen, Nukleolen und die Veränderungen im Protoplasma bei der Karyokinese", 'Beitr. Biol. Pflanz.', xl.
- Macallum, A. B. (1895).—"On the distribution of assimilated iron compounds other than haemoglobin and haematin, in animal and vegetable cells", 'Quart. Journ. Micr. Sci.', 33.
- Merejkovsky, C. de (1880).—"Sur l'origine et le développement de l'œuf chez la méduse *Euclope* avant la fécondation", 'C. R. Acad. Sc.', 90.
- (1883).—"Histoire du développement de la méduse *Obelia*", 'Bull. Soc. Zool. France', 8.
- Metschnikoff (1886).—"Embryologische Studien an Medusen". Wien.

- Moll, J. W. (1893).—"Observations on Karyokinesis in *Spirogyra*", 'Verh. d. K. Akad. van Wetensch. Amsterdam', 1, no. 9.
- Montgomery, T. H. (1898).—"Comparative cytological studies, with especial regard to the morphology of the nucleolus", 'Journ. Morph.', 15.
- Mulsow, K. (1911).—"Chromosomenverhältnisse bei *Ancyracanthus cystidicola*", 'Zool. Anz.', 38.
- (1912).—"Der Chromosomencyclus bei *Ancyracanthus cystidicola*", 'Arch. Zellf.', 9.
- Schazel, J. (1910 i).—"Die Eibildung der Meduse *Pelagia noctiluca*", 'Pér. et Less. Festschr. R. Hertwig'. Jena, 1.
- (1910 ii).—"Die Bedeutung des Chromatins nach Untersuchungen an Metazoenzellen", 'Verh. VIII Internat. Zool. Kongr.'
- (1911).—"Das Verhalten des Chromatins bei der Eibildung einiger Hydrozoen", 'Zool. Jahrb. Abt. Anat. Ontog.', 31.
- Schneider, A. (1873).—"Untersuchungen über Platyhelminthen", 'Bericht. oberhess. Gesellsch.', 14.
- Stout, A. B. (1912).—"The individuality of the chromosomes and their serial arrangement in *Carex aquatilis*", 'Arch. Zellf.', 9.
- Strangeways, T. S. P. (1924).—"Tissue culture in relation to growth and differentiation'.
- Stschelkanowzew, J. (1906).—"Die Entwicklung von *Cunina proboscidea* Metschn.", 'Mitth. Zool. Stat. Neapel', xvii.
- Tamura, O. (1923).—"Morphologische Studie über Chromosomen und Zellkerne", 'Arch. Zellf.', 17.
- Tischler, G. (1910).—"Untersuchungen über die Entwicklung des *Bananenpollens*", *ibid.*, 5.
- Trinci, G. (1905).—"L'oocite degli Idroidi durante il periodo di crescita", 'Monit. Zool. Ital.', 16.
- (1906).—"Studii sull' oocite dei Celenterati durante il periodo di crescita", 'Arch. Anat. Embr. Firenze', 5.
- Went, F. A. F. C. (1887).—"Beobachtungen über Kern- und Zelltheilung", 'Ber. Deutsch. Botan. Gesell.', 5.
- Wilson, E. B. (1901).—"A critical study of artificial parthenogenesis in sea-urchin eggs", 'Arch. Entw. Mech.'

The Histology of the Alimentary Tract of the Plaice (*Pleuronectes platessa*).

By

Ben Dawes, A.R.C.S., D.I.C.,

Research Assistant to the Marine Biological Association at Plymouth.

With 12 Text-figures.

CONTENTS.

	PAGE
INTRODUCTION, MATERIAL, AND METHODS	243
HISTORICAL	244
General Morphological Notes	246
Histology	248
Buccal Cavity and Pharynx	248
Oesophagus	252
Stomach	254
Duodenum and Intestine	259
Pyloric Caeca	262
Rectum	264
DISCUSSION	266
ACKNOWLEDGEMENT	272
SUMMARY	272
LITERATURE LIST	273

INTRODUCTION, MATERIAL, AND METHODS.

THE research work about to be described was undertaken as a preliminary to a study of the physiology of digestion in the fish named. Histological examination of tissues must necessarily precede a study of the functions of these tissues, especially so where one of the functions happens to be secretory. This is not realized as fully as one would incline to believe, and published accounts of the physiology of digestion of fishes are met with where the histology of the alimentary tract of the particular fishes is at present unknown.

Histological work calls emphatically for freshly preserved material, particularly in the case of a fish. Materials landed as 'trawled fish' in the economic sense are of little or of no use. The fish used in the work to be described were freshly trawled in Cawsand Bay and were either brought directly into the laboratory in the living condition or placed in the constructed 'pond' at Pier Cellars, into which each tide flows, to be taken out alive when required. At the time of fixation, therefore, all tissues were in the freshest condition.

Bouin's fluid was used exclusively and the state of fixation was generally very good. Xylol or clove oil or both served as clearing agents and the usual method of paraffin-wax embedding was employed. Slides were treated with soap and water, chromic acid, and 70 per cent. alcohol with a little added ammonia in turn, and were stored in 70 per cent. alcohol. Sections were fixed by the egg-albumen and glycerine method after stretching on a water bath.

The stains used were Mallory's triple, Delafield's and Ehrlich's haemotoxylin, mucicarmine, and borax-carmin with picro-nigrosin as counterstain. Two at least, often three, of these stains were used on each piece of tissue sectioned with a view to verifying the presence of structures within the cells.

Tissues were taken at various stages during the digestive processes, so as to render clearly the histological changes consequent upon digestion. This was made possible by the facilities to hand for maintaining fish alive.

HISTORICAL.

It is quite impossible in a short historical account to do justice to the work that has been carried out on the structure of the digestive tract of fishes. The workers have been numerous, particularly during the middle and latter parts of last century. Macroscopic researches commenced about the beginning of the century, or rather earlier, when Monro (1795), Home Everade (1814), Rathke (1824), and others all contributed substantially to current knowledge of the gross structures of the alimentary system.

With the institution of the microscope came the work of Rathke (1841) on *Amphioxus*, and that of Müller (J.) (1843) on *Myxine*, to inaugurate another and more complete series of researches on the microscopical structure of what had hitherto been conceived of as gross structures. Agassiz and Vogt (1845) prepared sections of the stomach of the trout and observed that what have been termed 'anfractuosités de la muqueuse' appeared as a 'rouleau' in the form of a club and consisted of two types of 'cellules', round and cylindrical ones.

Without in the slightest intending to disparage the sincere and careful endeavours of these early workers, one must observe that their attempts did not invariably meet with success. Valatour (1861), in his researches on the teleost fishes, failed to realize the importance of using fresh material, with the result that his work was to some extent disregarded, even discredited, by some of his immediate successors. Many of his findings received support, however, when the work of Edinger was published. Edinger (1877) enjoyed many distinct advantages, the refinement of histological technique through sixteen years, and the examples and results of several workers on mammalian histology, men such as Heidenhain (1870) and Rollett (1871). He observed that the gastric glands of fishes differed histologically from those of mammals, showed that the structure of the pyloric caeca was very similar to that of the intestine, and remarked upon the absence of intestinal glands.

Macallum (1884) studied the alimentary canal of the 'ganoids', in which he noted and described a ciliated epithelium in the oesophagus and stomach. Pilliet (1894) concerned himself principally with the *Pleuronectidae*, on which family nothing relevant to this piece of research has appeared in the literature since, excepting the work of Cole and Johnstone (1901). The work of Pilliet will be mentioned and discussed in the more appropriate part of this paper. Yung (1899) gave a good account of the work previously carried out but overlooked that of Gulland (1898), which was probably in the press at his time of writing, and merely mentioned that of Stirling (1884). The latter worked on the herring, Gulland on the salmon, where

changes in the nature of the mucosa during spawning migrations were specially treated.

During the current century a set of three researches are on record: that of Sullivan (1907) on Elasmobranchs, recording a ciliated oesophageal epithelium, that of Greene on the king-salmon (1912), and a short paper by Vonk (1928), which is largely physiological. Thus, from the point of view of modern technique, the literature is by no means extensive.

General Morphological Notes.

The alimentary tract of the plaice is a fairly uniform tube which, *in situ*, is formed into a number of rather complicated folds. It is externally only partially differentiated into regions of which the following will be recognized in addition to the buccal cavity: pharynx, oesophagus, stomach, duodenum, intestine, and rectum. The tissue referred to as the pharynx is that situated between and slightly behind the pharyngeal teeth, the oesophagus being the posterior continuation of this tissue into the stomach. There is no external indication of the passage of the oesophagus into the stomach but if the alimentary tract is opened and the mucosa examined in a fresh condition, the line of demarcation is quite strongly shown, the gastric mucosal folds appearing distinctly different from the oesophageal ones, even to the naked eye. This line can be taken as being very slightly posterior to the posterior wall of the pericardium. That there is sufficient justification for segregating the pharynx and oesophagus will be evident when the histological differences have been described.

The oesophagus widens as it passes into the stomach and the latter organ attains its greatest diameter about the middle of its length, posterior to which it becomes more slender especially near the pyloric sphincter and valve. The free end of this valve projects into the intestine of course.

Three regions are recognized in the post-pyloric intestine, the duodenum, intestine, and rectum. The first two, which together form approximately half the entire length of the alimentary tract, are arbitrary divisions of the pre-rectal intestine, made

with a view to investigating separately anterior and posterior halves of this relatively long stretch of gut. The third region, the rectum, is relatively short, but the boundary between it and the intestine is a real one, as will be seen later.

Into the duodenum open the four very small and rudimentary (or vestigial) pyloric caeca and also the bile duct. Along the whole length of the duodenum and of the intestine, the ultimate factors of the portal vein, which penetrate the wall of the alimentary tract at indeterminate points, are embedded in loose glandular tissue. Similar tissue is found on the mesenteries carrying these blood-vessels and around the blood-vessels in the tissue of the liver. It represents the diffuse type of pancreas, which characterizes the teleost fish.

In the empty condition the rectum is not easily recognized as a separate region, but in the full condition towards the end of a meal it often appears very considerably distended, even though the intestine is completely empty. This fact immediately suggests the presence of a valve separating the two regions and examination proves that this is the case. The valve, the free end of which projects posteriorly, is quite as well developed as is the pyloric valve. It will be referred to as the intestino-rectal valve.

Although the relative lengths of the above-mentioned regions are doubtlessly subject to considerable variation, the following carefully made measurements of the series in a single fish, 29.0 cm. long, will impart some idea of their order :

	cm.
Length of whole tract, uncoiled, from lips to anus .	28.0
Lips to anterior margin of pharyngeal teeth (= buccal cavity)	4.0
Anterior margin of pharyngeal teeth to anterior end of stomach (= pharynx plus oesophagus)	2.8
Anterior end of stomach to pyloric valve	4.7
Pyloric valve to intestino-rectal valve (= duodenum plus intestine)	14.5
Intestino-rectal valve to anus (= rectum contracted) .	2.0

Histology.

The entire alimentary tract, with the exception of the pharynx, consists of the following layers commencing from without and proceeding towards the lumen :

- (1) Serosa : a thin layer of areolar tissue on which connective tissue-cells are superimposed.
- (2) Outer longitudinal muscular layer : a thin sheet of muscle-bundles having longitudinally disposed fibres.
- (3) Inner circular muscular layer : a relatively thicker sheet of transversely disposed fibres.
- (4) Sub-mucous coat : a layer of more or less loose areolar connective tissue in which are embedded numerous small blood-vessels and fine nerve-fibres.
- (5) Mucous membrane : a membrane of variable thickness according to the region to which it belongs and to the degree of distention of that region during digestion.

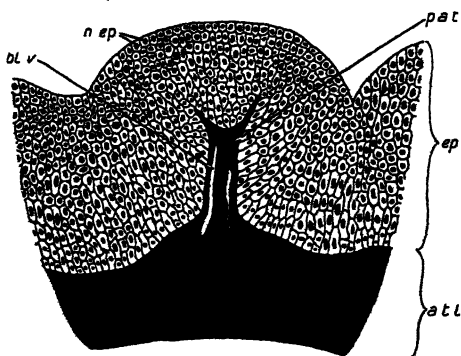
These layers, therefore, characterize the fish. They vary in certain features all along the tract. The muscular layers and the mucous membrane show the greatest histological variation regionally, the serosa and the sub-mucous coat being fairly constant in histological detail throughout. There is no trace of a muscularis mucosa, a stratum granulosum, or a stratum compactum in any part of the tract. In the description which follows, wearying repetition of names will be avoided as much as possible, mention being made chiefly of the histological variations met with in the regions included between the lips and the anus.

Buccal Cavity and Pharynx.

The mucous membrane of the buccal cavity consists of a stratified epithelium which rests upon a basement membrane. Below it is a layer of loose areolar tissue containing minute blood-vessels. The epithelium is gently corrugated, the folds running longitudinally and containing short processes of areolar tissue. In addition to these corrugations, microscopic papillae of connective tissue project into the epithelium at certain

indeterminate points (Text-fig. 1, *p.a.t.*), which papillae each contain small blood-vessels and in some cases a fine nerve-fibril also. The epithelium is about ten to twelve cells in thickness, the deeper cells being columnar with rounded extremities, while the superficial cells are polyhedral or round. Apparently there are no scale-like cells at or near the periphery. The nuclei are generally spherical or, in the basal cells, oval. Minute inter-cellular spaces appear to exist between the epithelial cells.

TEXT-FIG. 1.



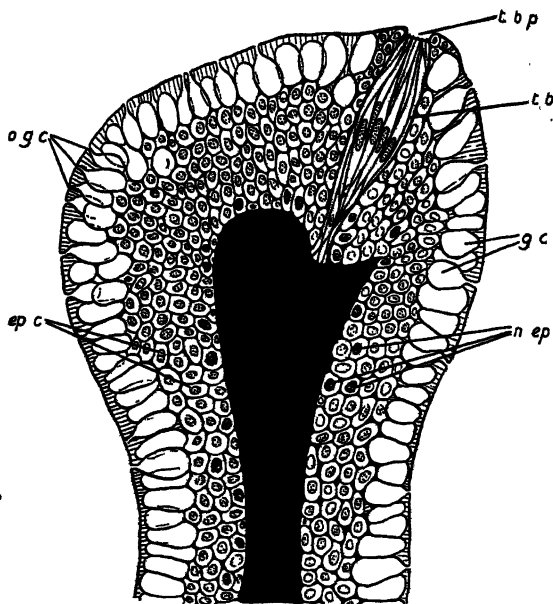
Vertical section of the mucous membrane of the buccal cavity. *a.t.l.*, areolar tissue-layer; *bl.v.*, blood-vessel; *ep.*, epithelial layer; *n.ep.*, nuclei of the epithelial cells; *p.a.t.*, papilla of areolar tissue passing into the epithelium. ($\times 150$.)

Goblet-cells are exceedingly rare, but some few are found as perfectly isolated units. These are spherical or oval in form, appear to lack the tail-like process usually found at the base of goblet-cells, and they open to the exterior by a relatively wide pore. Structures resembling the taste-buds of mammals occur but extremely rarely. These are a characteristic feature of the mucous membrane of the pharynx (*vide infra*), and for all practical purposes they can be neglected in the case of the buccal cavity.

Considerable differences are seen in the pharynx. The epithelium, like that of the buccal cavity, is stratified but consists of fewer cell-layers (Text-fig. 2, *ep.c.*). It is intensely folded so that the lumen is reduced to a series of much-branched canals

in the contracted condition. The main lumen is preserved, however, by virtue of the fact that the most superficial folds are extensive transversely. Finger-like processes of the areolar tissue-layer project into the folds no matter how minute these

TEXT-FIG. 2.



Transverse section passing through a mucosal fold of the pharynx.

Areolar tissue-layer rendered in black. *ep.c.*, epithelial cells; *g.c.*, goblet-cells; *n.ep.*, indeterminate nuclei of epithelial cells rich in chromatin; *o.g.c.*, openings of goblet-cells; *t.b.*, 'taste-bud'; *t.b.p.*, shallow pit in the epithelium seen at the periphery of the taste-bud. ($\times 200$.)

happen to be. Goblet-cells are extraordinarily numerous, occurring in an unbroken sequence throughout the extent of the mucosa, so that one never succeeds in locating an area devoid of them, or even where the regular sequence is broken. They are most numerous in the deeper folds, being two, three, or more deep, while in the superficial folds which bound the lumen they usually form shallow patches one, or at most two, goblets deep (Text-fig. 2, *g.c.*). In any situation they are not found typically

at the surface but usually lie more deeply situated, the goblet communicating with the lumen of the pharynx by a conspicuous neck and a small pore. The goblets are oval except where laterally compressed due to great crowding and where underlying other goblet-cells, when they are spherical. The basal process is not filamentous, but appears like a small accretion on the base of the goblet. The stratified nature of the epithelium probably accounts for the development of this unusual type of cell.

Near the base of the pharyngeal epithelium in the superficial folds bounding the lumen cells are found, the nuclei of which show conspicuous heavily stained chromatin masses. They are less conspicuous in the deeper folds of the mucosa, where the epithelium is thinner and composed of fewer cells, despite the fact that goblet-cells are more numerous. These appear to be actively dividing cells of the epithelium (Text-fig. 2, *n.ep.*).

In the peripheral part of the epithelium of the pharynx, and especially in the superficial folds bounding the main portion of the lumen, small clusters of cells resembling taste-buds occur. The individual cells of each cluster are of an elongate spindle-shape, possess oval nuclei, and are closely packed together (Text-fig. 2, *t.b.*). The spindle-cells reach almost to the basement membrane, from which they are separated by columnar cells. Towards the periphery they terminate each in a fine, deeply staining process, which projects into a pit-like depression in the mucosa (Text-fig. 2, *t.b.p.*). The spindle-cells, which on analogy with similar cells in the mammal, can be regarded as gustatory cells, do not appear to possess branched basal processes such as are found in some cells of this type.

The musculature of the pharynx consists largely of a thick circularly disposed band of striated fibres, which is continuous with the circular musculature of the oesophagus and stomach. In addition to this, there exist a number of loosely arranged muscle-bundles, longitudinally disposed but slightly oblique, which form a second series within the areolar tissue-layer. These bundles also consist of striated fibres. There is no continuum of the outer longitudinal musculature of the oesophagus and stomach in this region.

Oesophagus.

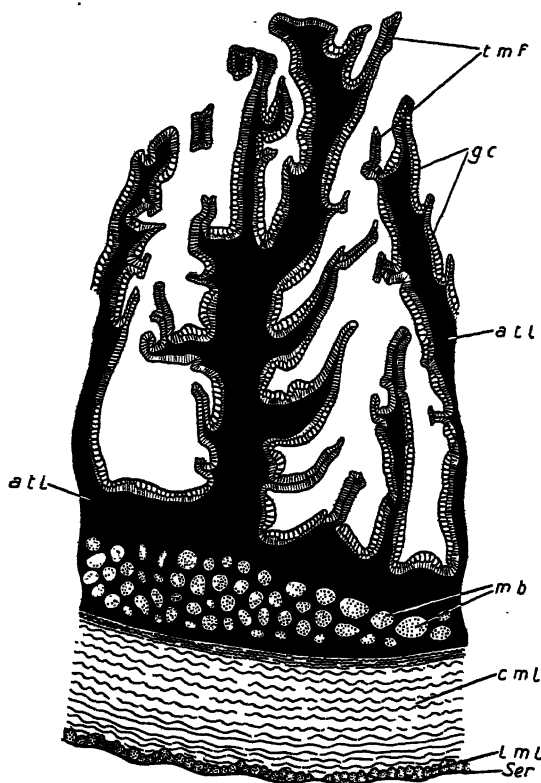
The serosa is an extremely thin layer of areolar connective tissue continued externally into the tissue of the mesenteries and internally into the connective tissue binding together the muscle-bundles immediately beneath it (Text-fig. 3, *ser.*). It is penetrated at indeterminate points by small blood-vessels and fine nerves as these enter the tissue of the alimentary tract. The outer longitudinal musculature does not take the form of an unbroken ring, being represented by irregularly disposed bundles (Text-fig. 3, *l.m.l.*), while the inner circular muscular layer is of enormous thickness, at least ten times the thickness of the inner layer (Text-fig. 3, *c.m.l.*). All muscle-fibres are of the striated type, as in the pharynx. The loosely arranged muscle-bundles observed in the areolar tissue-layer of the pharynx are continued throughout the oesophageal region as even more scattered bundles forming crescentic masses opposite the mesenteries (Text-fig. 3, *m.b.*).

The mucosa is folded to an even greater degree than in the pharynx, or incidentally in any other part of the tract. The folds form primary and secondary series as regards branching, and transverse sections of this region show a maze of folded mucosa and occluded lumen. A small and relatively simple fold is shown in Text-fig. 3. Wider channels of lumen remain centrally to form a stellate canal into which the smaller channels of lumen open. The epithelium is no longer stratified but where unmodified by the presence of goblet-cells consists of a single layer of columnar, but not very tall, cells with oval nuclei which take up the major part of the volume of their cells. These unmodified regions are to be found at the tips of the branches of the mucosa (Text-fig. 3, *t.m.f.*). Goblet-cells are numerous but occupy only the depressions of the mucosa, where they form extensive patches which contrast strongly with the unmodified papilla-like tips. They form only a single series and are usually laterally compressed due to crowding. The nuclei are to be found embedded in a crescentic mass of cytoplasm at the base of the goblets. Below the goblet-cells is a 'cushion' of small rounded cells, the only suggestion of stratification in the

epithelium. With the fixative employed, the contents of the goblets present a reticular appearance.

Although occasional leucocytes are found between the un-

TEXT-FIG. 3.

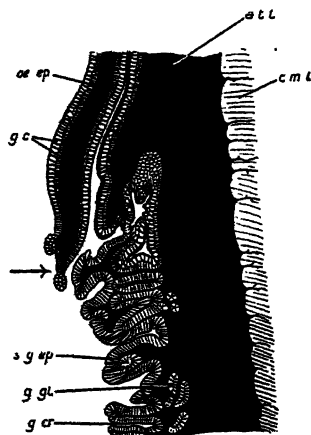


Transverse section of a portion of the oesophagus, showing the intense degree of mucosal folding. *a.t.l.*, areolar tissue-layer; *c.m.l.*, circular muscular layer; *g.c.*, goblet-cells of the epithelial layer; *l.m.l.*, longitudinal muscular layer; *m.b.*, loosely arranged, longitudinally disposed muscle-bundles within the areolar tissue-layer; *ser.*, serosa; *t.m.f.*, tips of the mucosal folds lacking goblet-cells. ($\times 40$.)

modified cells of the epithelium, it seems unlikely that such isolated units can materially aid food absorption. The histologi-

cal features enumerated above for the pharynx and oesophagus, the intensely folded mucosa, well-developed striated musculature, abundance of mucous-producing cells, tend to demonstrate that this part of the alimentary tract is principally, if not solely,

TEXT-FIG. 4.



Longitudinal section through the junction of oesophagus and stomach. The arrow shows approximately the line of demarcation, oesophagus above, stomach below. *a.t.l.*, areolar tissue-layer; *c.m.l.*, circular muscular layer; *g.c.*, goblet-cells; *g.cr.*, crypt into which gastric gland opens; *g.gl.*, gastric gland; *oe.ep.*, oesophageal epithelium; *s.g.ep.*, superficial epithelium of the stomach. ($\times 50$.)

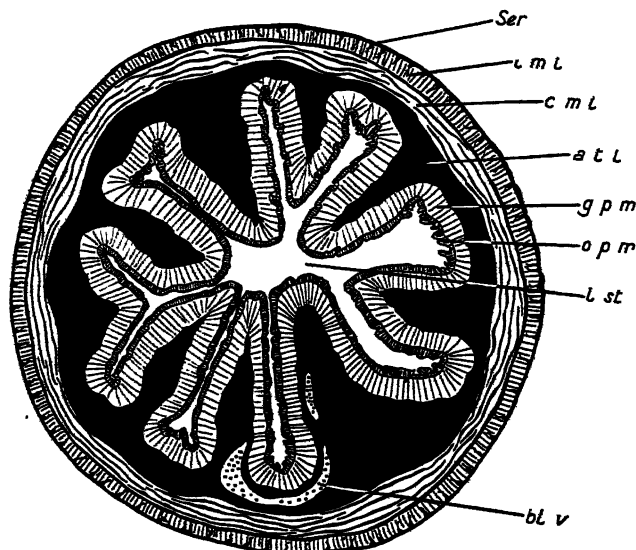
concerned with a facile swallowing of large morsels of food, which may be selected by means of the gustatory organs.

Stomach.

Although, as has been stated, there is no external morphological indication of the passage of the oesophagus into the stomach, yet there is a fairly sharp line of demarcation between the organs histologically. The transition from oesophagus to stomach is marked first of all by the loss of the complex mucosal folds, in place of which are seen in the stomach seven or eight large and more or less equal longitudinal folds. In the contracted condition of the organ, these are approximated so that the lumen

is resolved into seven or eight radiating channels of narrow calibre (Text-fig. 5, *l.st.*). It is also marked by the disappearance of goblet-cells (Text-fig. 4, *s.g.ep.*). A longitudinal section shows the abrupt nature of the change and also that the oesophageal

TEXT-FIG. 5.



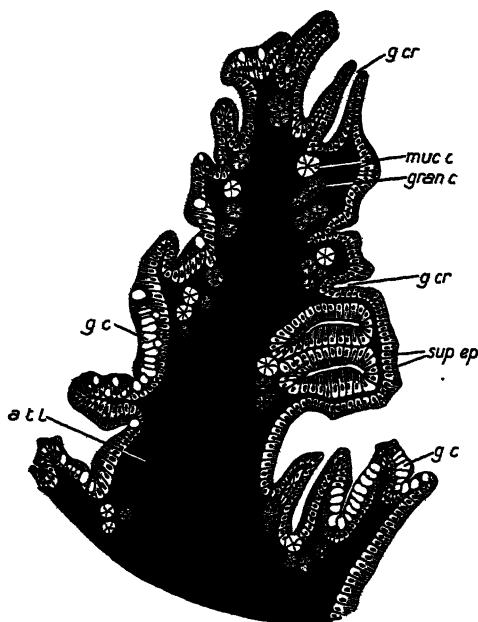
Diagrammatic transverse section of the stomach in the empty condition. *a.t.l.*, areolar tissue-layer; *bl.v.*, blood-vessel; *c.m.l.*, circular muscular layer; *g.p.m.*, deep, glandular part of the mucosa; *l.m.l.*, longitudinal muscular layer; *l.st.*, lumen of stomach; *o.p.m.*, superficial part of mucosa containing crypts; *ser.*, serosa. ($\times 25$.)

epithelium is continued into the superficial epithelium of the stomach (Text-fig. 4, *oe.ep.*, *s.g.ep.*).

The gastric mucosa varies in thickness in different parts of the organ, being much thicker in the middle and posterior parts than anteriorly. The thickness is directly due to the degree of folding, i.e. to the degree of development of the gastric glands. These are both deeper and more numerous in the mid-stomach and posteriorly. Anteriorly, they are very shallow (Text-fig. 6), although the crypts are well marked, and here resemble oeso-

phageal folds (Text-fig. 6, *g.cr.*). The glands are of the simple tubular type, but appear to be slightly branched in the mid-stomach and posteriorly, and are closely aggregated. The superficial epithelium is formed into a series of pit-like depressions

TEXT-FIG. 6.

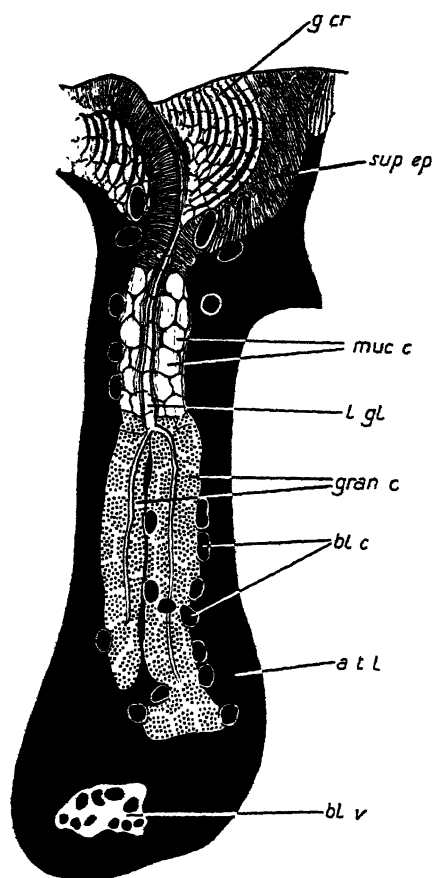


Transverse section passing through a mucosal fold near the anterior extremity of the stomach. Indicating the shallow and simple nature of the gastric glands. *a.t.l.*, areolar tissue; *g.c.*, goblet-cells; *g.cr.*, crypt leading into lumen of gland; *gran.c.*, granular cells at base of gland tubule; *muc.c.*, mucus-producing cells forming the neck of the tubule; *sup.ep.*, superficial epithelium. ($\times 125$.)

or crypts which lead into the glands. This superficial epithelium consists of columnar cells rather taller than those of the unmodified tips of the mucosal folds in the oesophagus. The nuclei are oval and difficult to stain during a prolonged resting period, e.g. during the period of the 'empty stomach'. The cell cytoplasm behaves in a striking manner to stains. Near the periphery is a narrow blue-stained zone (with Mallory); rather

more deeply situated is an equally narrow red-stained zone, and in the middle region of the cell just outside the nucleus is a broad

TEXT-FIG. 7.



Vertical section of a gastric gland from the mid-stomach. *a.t.l.*, areolar tissue; *bl.c.* and *bl.v.*, blood-corpuscles and vessel; *g.cr.*, crypt (rendered in perspective as seen in a relatively thick section); *gran.c.*, granular cells; *l.gl.*, lumen of gland; *muc.c.*, mucus-producing cells; *sup.ep.*, superficial epithelium lining crypt. ($\times 300$.)

blue-stained mass. This latter is also visible with borax-carmin, picro-nigrosin staining as a greyish mass. With

mucicarmine one obtains no stain except in the peripheral part of the cells lining the crypts. These superficial cells are probably slightly mucus-producing, but are not typically so.

The gastric glands consist of cubical cells of two types. In the neck of the glands immediately below the crypts occur cells which, during a prolonged resting phase, appear devoid of granules and unstained or faintly blue with Mallory or Delafield's haematoxylin. During active digestion these cells show only a border behaving in the above manner, the basal part of the cell staining more deeply. With mucicarmine these cells stain reddish, so that they are undoubtedly mucus-producing cells. They can be compared to the mucoid cells scattered among the so-called peptic cells of the mammalian fundic gland. The basal part of the gland tubule consists of granular cells, which are probably the enzyme-secreting cells of the gland. There is no sign of cells resembling the oxyntic or parietal cells of mammals in any part of the gastric mucosa. The component cells of the glands are illustrated in Text-fig. 7, *sup.ep.*, *muc.c.*, *gran.c.*

Text-fig. 8 illustrates diagrammatically a hypothetical conception of the possible transition from the oesophageal type of mucosa to the gastric type. The intermediate type does not exist as shown in B, but an approximation to it is seen at the junction of oesophagus and stomach (Text-fig. 6, bottom, right). The homologous tissues are: (1) the tips of the oesophageal mucosal folds and the superficial epithelium of the stomach; (2) the goblet-cells of the oesophagus and the mucus-producing cells of the gastric tubule; and (3) the cushion of cells below the goblet-cells in the oesophagus and the granular basal cells of the gastric gland.

Near the pyloric sphincter the gastric glands become more shallow and opposite the sphincter, they disappear altogether while the crypts remain (Text-fig. 11, *sup.ep.*). The sphincter is merely the enormously thickened circular musculature. The muscles of the stomach consist of non-striated fibres, the transition from the striated type occurring near the anterior end of the organ. Here also the loosely arranged muscle-bundles in

the areolar tissue-layer disappear. In the mid-stomach the inner and outer muscular layers are of approximately the same thickness, the former gradually thickening near the pyloric sphincter. The pyloric valve contains muscles from both the above layers but largely from the inner one. Goblet-cells occur on each side of the valve although the types of mucosal folds differ,

TEXT-FIG. 8.

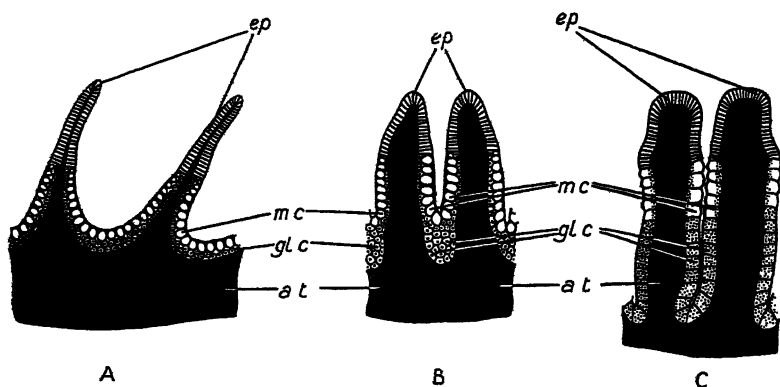


Diagram illustrating an hypothetical conception of the transition from the oesophageal type of mucosa to the gastric type. A, oesophagus; C, stomach, and B, hypothetical intermediate type. *a.t.*, areolar tissue; *ep.*, in A, epithelium of tips of mucosal folds passing through B to form the superficial epithelium continued into the crypts in C; *m.c.*, in A, goblet-cells forming the mucus-producing part of the gland tubule in C; *gl.c.*, in A, the 'cushion' of epithelial tissue beneath the goblet-cells in the mucosal troughs, in C, the granular cells at the bases of the tubules.

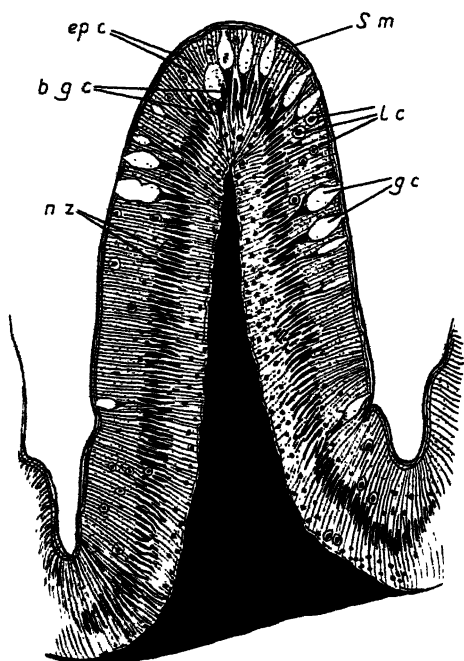
those on the anterior aspect to some extent resembling gastric crypts.

Duodenum and Intestine.

The mucosa of the duodenum and intestine is relatively simple in structure. In bulk it is gently corrugated into a large number of longitudinal folds which, unlike those of the oesophageal mucosa, are unbranched. During the period when the tract is empty, slight transverse folds also occur. The folds to some extent simulate villi, while the mucosal depressions give

a false impression of crypt-like glands, especially in material fixed during a resting phase when the organ is empty. During digestion the intestine is greatly distended and the folds smoothed out. That villi and glands do not occur is indicated by the lack of histological differentiation in the mucosa, which consists of

TEXT-FIG. 9.

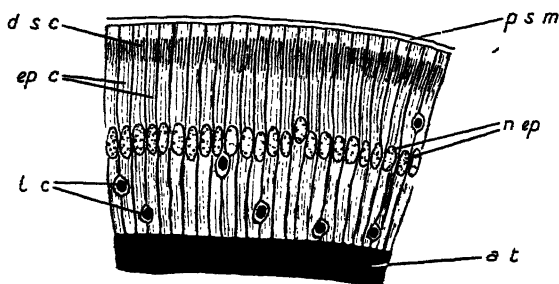


Transverse section of a mucosal fold in the duodenum, fixed during a prolonged resting phase. Areolar tissue rendered in black. *b.g.c.*, filamentous basal part of the goblet-cells; *ep.c.*, columnar cells of epithelium; *g.c.*, goblet-cells; *l.c.*, leucocytes; *n.z.*, spindle-shaped zone containing nucleus; *s.m.*, darkly staining border of epithelium. ($\times 250$.)

tall and extremely slender columnar cells interspersed with goblet-cells. The nuclei are small and oval and, like the nuclei of the cells forming the gastric tubules, are difficult to stain during a prolonged resting phase, when the cytoplasm stains more deeply. Tissue fixed at the end of such a phase in Bouin's

fluid and stained in section with Delafield's haemotoxylin shows a deep-blue spindle-shaped mass about three times the length of the nucleus situated about the middle of the cell. In material fixed during active digestion the only parts of the nucleus taking up stain are the periphery and a fine chromatin network. The outer half of each cell also stains less deeply and the margin instead of appearing heavily stained is now quite pale. At the same time a rather darker mass appears just below the pale

TEXT-FIG. 10.



Vertical section of the intestinal mucosa, fixed during active digestion of food. *a.t.*, areolar tissue; *d.s.c.*, rather more deeply staining cytoplasm; *ep.c.*, columnar cells of epithelium; *l.c.*, leucocytes; *n.ep.*, nuclei of epithelial cells; *p.s.m.*, margin of epithelium, now pale-staining. ($\times 500$.)

border. Text-fig. 9 shows the epithelium during a resting phase, Text-fig. 10 during active digestion.

Numerous isolated goblet-cells occur in the epithelium. They are not found in patches as in the oesophagus and often relatively large areas of mucosa are seen to be devoid of them. They differ from the corresponding cells in the pharynx and oesophagus in that they are smaller, regularly oval, and possess a filamentous basal portion which contains the nucleus (Text-fig. 9, *g.c.*, *b.g.c.*).

Leucocytes are to be found between the epithelial cells of both duodenum and intestine at all times. Their small rounded nuclei, which stain blackish with Delafield's haemotoxylin, render them conspicuous (Text-fig. 9 and Text-fig. 10, *l.c.*).

They appear in all zones to the margin of the epithelium, which apparently they never penetrate. If anything they appear in slightly greater numbers during digestion, although there is no marked change and it appears unlikely that they play any considerable part in this process.

Processes of the areolar tissue-layer pass into the mucosal folds and carry small blood-vessels. There is no sign of a central lacteal, so that the folds are definitely not villi. In view of the fact that the muscularis mucosa is absent, lacteals homologous with those of mammals could not exist in the plaice, since this layer in part forms the wall of these structures. It is probable that the spaces in the areolar tissue serve as lacteals in this fish.

The musculature of the duodenum and intestine consists of equally thick circular and longitudinal layers. The fibres are of the non-striated type of course. Between the two layers occur the nerve-fibres and cell-clusters of the plexus of Auerbach, which appears very prominently in all sections of these regions. In material treated by the usual methods of histological technique there is no sign of a ganglionated plexus comparable with that of Meissner, which is found in the sub-mucous coat of the mammalian intestine.

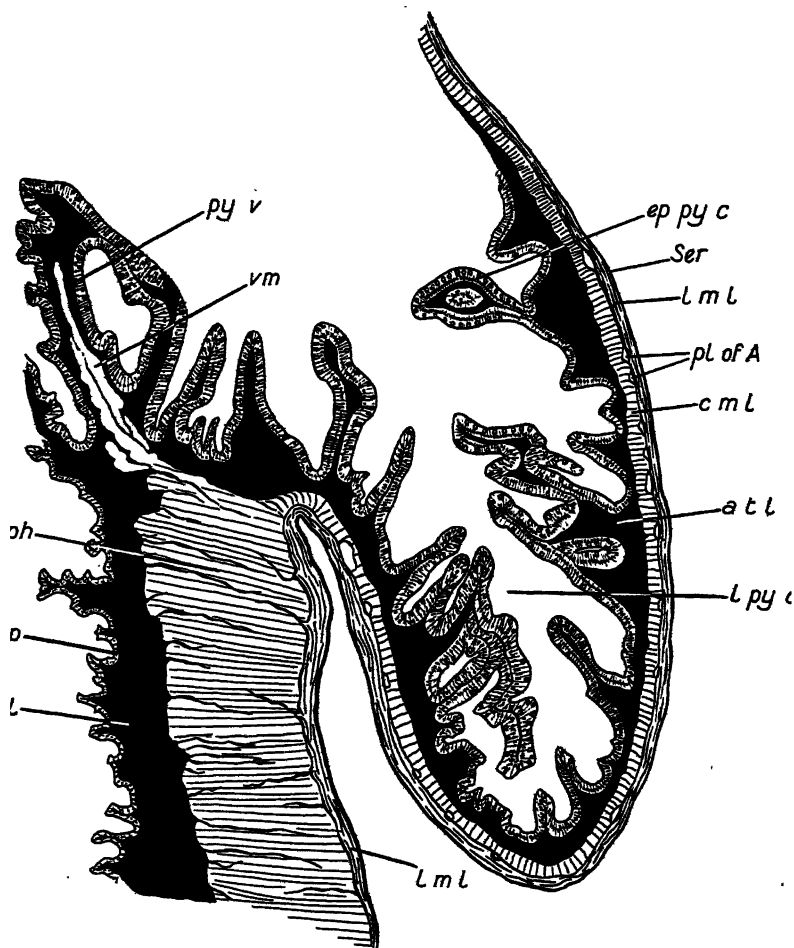
The serosa of the intestine is much thicker than is that of more anteriorly situated regions of the alimentary tract.

Pyloric Caeca.

The histological structure of the pyloric caeca is similar to that of the intestine. The muscular layers are thinner and the mucosa is rather more considerably folded, although the folds are simple like those of the duodenum and intestine. The increased degree of folding appears to be due entirely to the almost spherical form of the caeca, which, as has already been stated, are very small. The lumen of each caecum is necessarily reduced but by no means obliterated, so that it communicates freely with the lumen of the duodenum (Text-fig. 11, *l.py.c.*). Thus the caeca are practically pockets of the intestine into which food can readily pass.

The epithelium is similar to that of the duodenum, consisting

TEXT-FIG. 11.



Longitudinal section passing through the pylorus and a pyloric caecum. *a.t.l.*, areolar tissue-layer; *c.m.l.*, circular muscular layer; *ep.py.c.*, epithelium of caecum; *l.m.l.*, longitudinal muscular layer; *l.py.c.*, lumen of caecum; *p.sph.*, pyloric sphincter; *pl. of A.*, nerve-fibres of Auerbach's plexus; *py.v.*, pyloric valve; *ser.*, serosa; *sup.ep.*, superficial epithelium of stomach (note the absence of glands in this region); *v.m.*, process of circular muscles contributing to the pyloric valve. ($\times 30$.)

of tall and slender columnar cells interspersed with goblet-cells. Leucocytes are found between the columnar cells just as commonly as is the case in the duodenum and intestine. Between the muscular layers the plexus of Auerbach is conspicuous also (Text-fig. 11, *pl. of A*). In fact all the characteristic features of the post-pyloric intestine are repeated in the structure of the caeca, which can thus be regarded as an integral part of the alimentary tract in this region.

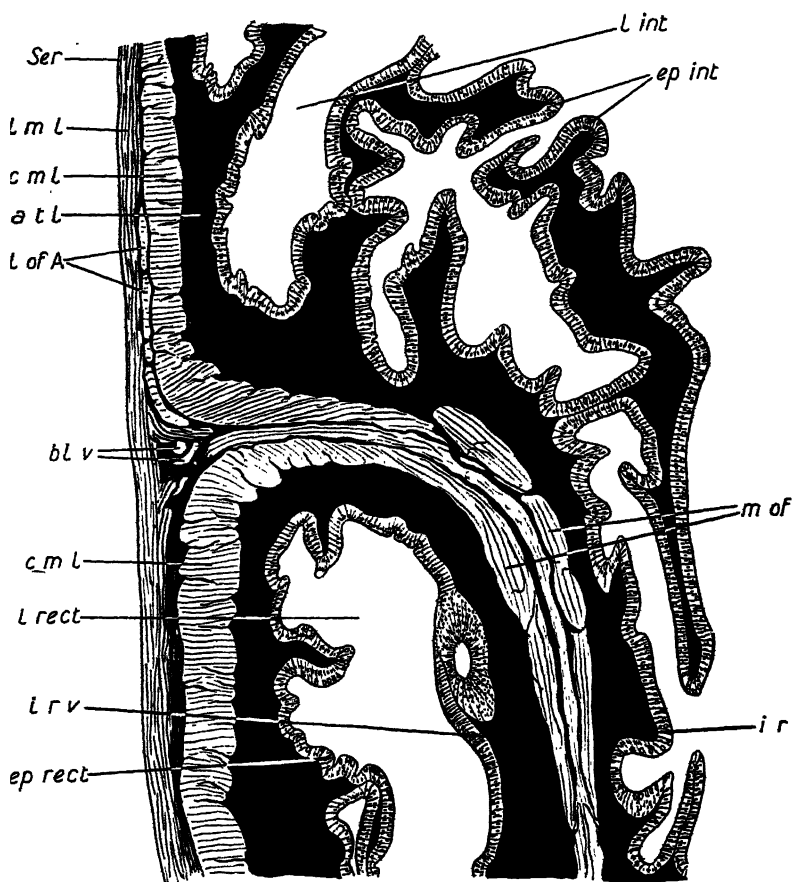
Rectum.

The boundary between the intestine and the rectum is marked by the intestino-rectal valve, as was stated earlier. This is quite as well developed as the pyloric valve which marks the boundary between the stomach and the duodenum. It differs from this latter valve in that its musculature is derived equally from that of the tract on each side of it (Text-fig. 12, *m. of v.*). The circular muscles of both intestine and rectum become slightly thicker near the point of origin of the valve and pass entirely into it, accompanied by a process of the longitudinal muscular layer. The nerve-fibres of Auerbach's plexus also pass into the tissue of the valve. The mucosa is of the intestinal type on both faces (Text-fig. 12, *ep.int.*, *ep.rect.*), and there are more goblet-cells on the rectal face. The free end of the valve projects posteriorly.

The mucosa of the rectum is similar to that of the intestine, the chief differences being a greater degree of folding in the closed condition and more numerous goblet-cells. These latter form an almost continuous sequence in some areas of tract and are of the same type as the corresponding cells of the intestine. Leucocytes occur between the columnar cells of the epithelium almost as commonly as in the intestine and at all times.

The musculature of the rectum is much thicker than that of the preceding part of the alimentary tract. The inner, circular layer is more than twice as thick, the outer, longitudinal layer almost twice as thick as the corresponding layers in the intestine. The inner layer becomes suddenly thickened near the anus,

TEXT-FIG. 12.



Longitudinal section of the junction of intestine and rectum. *a.t.l.*, areolar tissue-layer; *bl.v.*, blood-vessel; *c.m.l.*, circular musculature; *ep.int.*, intestinal epithelium; *ep.rect.*, rectal epithelium; *i.r.v.*, intestino-rectal valve; *l.int.*, lumen of intestine; *l.m.l.*, longitudinal musculature; *l.rect.*, lumen of rectum; *m. of v.*, musculature of valve; *pl. of A*, Auerbach's plexus; *ser.*, serosa. ($\times 40$.)

where it forms the anal sphincter. Here, the mucosal folds are very numerous and very deep, while the goblet-cells completely disappear.

DISCUSSION.

When a consideration is made of the structure of the alimentary tract in the various groups of fishes, many histological differences are found. The majority of these are bound up with gross morphological differences such as the types of stomach, the presence or absence of pyloric caeca or of a spiral intestinal valve and others. But differences occur apart from these. Macallum (1886) observed a ciliated epithelium in the oesophagus of *Amia*, *Lepidosteus*, and *Acipenser*, and even in the stomach of the first named. Sullivan (1907) describes a similar epithelium in the oesophagus of the Elasmobranchs. It appears that such an epithelial layer does not occur at all in the Teleostei as neither Greene (1912) nor Stirling (1884) observed it in the king-salmon and herring respectively. Gulland (1898) unfortunately omits a consideration of this region in the salmon, but in the plaice the present research shows that cilia are absent from every part of the tract. Nor did Pilliet (1894) observe a ciliated epithelium in any of the Pleuronectidae he studied.

Although there is no external indication of the passage of the oesophagus into the stomach in the plaice, there is, as we have seen, a definite, sharply defined histological boundary between the two regions. The mucosa of the oesophagus with its much-branched and complicated longitudinal folds and abundance of goblet-cells passes abruptly into a glandular stomach devoid of such folds and cells. Cole and Johnstone err in remarking that the oesophagus is sharply defined from the stomach by the strongly developed transverse musculature at the proximal end of the latter region. On the contrary, the immense circular muscles of the oesophagus diminish in thickness gradually in passing towards the mid-stomach. There is no abrupt change in thickness at any point. In passing it might also be added that the feebly developed longitudinal musculature consists of striated fibres and not of non-striated elements, as these writers maintain. They did not observe the loosely arranged muscle-bundles in the areolar tissue-layer.

The oesophagus of the herring is abundantly beset with goblet-

cells, between the upper ends of which Stirling also found small triangular cells intercalated. Greene did not observe goblet-cells in the king-salmon but instead found cells with basal nuclei and clear outer margins, which he regards as mucus-producing cells. Goblet-cells are very numerous in the oesophagus of the plaice, but there is no sign of an intercalated series of triangular cells, although occasional cells are found in this situation. These are explained when it is considered that cells below the periphery develop into goblet-cells in the depths of the folds where the epithelium is to some extent stratified. Unmodified cells, therefore, can remain in a superficial position and in other positions without forming a definite layer.

Only a few workers have differentiated between the pharynx and the oesophagus, probably because the two regions together form only a relatively short length of tract. Three features justify one in considering the pharynx as a definite region: the absence of longitudinal musculature, the definitely stratified epithelium with its abundance of goblet-cells, and the presence of structures referred to as taste-buds. I have not met with any hitherto published account of the occurrence of taste-buds in the pharynx of the teleost fish, but Macallum (1886) describes such structures in the oesophageal mucosa of *Acipenser*, remarking on the rare occurrence of these in a similar situation in the higher vertebrates. This leads him to the conclusion that the piscine and mammalian oesophageal regions are not homologous. Comparison of these regions in the plaice and the mammal is not particularly yielding, but it is conceivable that the deep folding of a mucosa richly supplied with goblet-cells might lead to the development of definite mucous glands in the folds, thus removing the necessity for superficial goblet-cells. If mucous glands of mammals have developed in this way, then the pharynx of the fish, possessing taste-buds as well as incipient mucous glands, would correspond to the similarly named region in the mammal plus adjacent regions such as the epiglottis and the tongue, i. e. the structures bearing the gustatory organs.

From the description given, it seems clear that the functions of the oesophagus and pharynx are gustatory, mucus-producing,

and the facilitating of rapid and efficient swallowing action. The absence of definite glands and specialized cells suggests that these regions possess little or no secretory power apart from that of mucus-production.

Heidenhain (1870) discovered two kinds of cells in the tubules of the gastric glands of mammals. These are now referred to as central and parietal or oxyntic cells. Edinger (1877) made a detailed study of the histology of the digestive tract of fishes and found that only one type of cell occurs in the tubules and that this type was neither central nor parietal. Gulland (1898) recognized two kinds of epithelium in the gland tubule of the salmon, but agreed that the parietal type of cell is non-existent. The two epithelial types he refers to as intermediate and zymoin-producing, the former being found in the neck of the gland while the latter forms the base of the tubule. He remarked on the appearance of a clear outer hem in the intermediate cells. Green (1912) says, 'There is no intermediate structurally intermediate type of neck-cell in the king-salmon as suggested by Gulland for the Atlantic salmon'. He believes that this type of cell is merely 'a foreshortened type of cylindrical cell'. But in the plaice two distinct types of cell occur in the gland tubule and are most evident during a prolonged resting phase. In the neck of the gland, cells occur which are only faintly stained in ordinary preparations but which are undoubtedly stained with mucicarmine, thus being mucus-producing cells. At the base of the tubule the cells are very granular and undoubtedly are enzyme-producing cells. The mucous cells are inconspicuous, relatively, during digestion, and it is suggested here that Greene probably overlooked them as a result of sectioning material fixed during digestive phases when they were not evident types. Gulland, being chiefly concerned with the period of the spawning migration, would use material fixed during a prolonged resting phase when, in the plaice at least, the cells are best demonstrated. Stirling (1884) also observed clear cells apparently filled with mucus in the ducts of the gastric glands of the cardiac sac of the herring, but did not emphasize this finding.

It appears that all workers are agreed that parietal or oxyntic

cells do not exist in fish, and as these cells are deemed hydrochloric acid producing the question of the source of the acid is still unsettled. Stirling, remarking on this fact, conceives it possible that the superficial cells perform more than one function and produce both the acid and mucus as well. In the plaice the cells of the neck of the tubules are undoubtedly the agents responsible for the production of mucus, being possibly derived from the goblet type of cell. The superficial cells, which are not typical mucus-producing cells, are probably in some way connected with the production of the acid found in the stomach during digestion. Gulland apparently under-estimated the importance of the cells in the neck of the tubule.

In the king-salmon gastric glands do not occur near the pyloric valve. Similarly in the plaice the region opposite the pyloric sphincter is devoid of glands although possessing crypts. According to Macallum, however, in the pyloric region of *Acipenser*, 'the majority of the cells are open peripherally; from this and from their being swollen they bear a resemblance to goblet-cells.' Thus, if the fixation of Macallum's material was all that could be desired, the absence of goblet-cells from the stomach is not general in fishes.

Mention must now be made of the work of Pilliet (1894). He investigated the stomach of the *Pleuronectidae*, using the turbot chiefly but also making use of the sole, the dab and the plaice. In the last two he was most unfortunate, and referring to these he says, 'Enfin le Carrelet nous a offert une exception qui mérite d'être signalée. L'estomac était vide, et nous avons eu beau multiplier les coupes de la muqueuse après la fixation par l'alcool, les glandes gastriques nous ont échappé.' And again, 'Pour la Limande, la même difficulté s'était présentée, mais nous avons réussi à tomber enfin sur la région glandulaire.' He later adds that after mounting supposed sections of the stomach of the plaice they invariably found that they had missed the stomach region, having obtained sections of either the oesophagus or the region of the pylorus. His explanation cannot be overlooked. He says, 'Il faudrait donc conclure que les glandes diminuent de nombre et de volume pendant certaines périodes,

pour reprendre champ pendant certaines autres.' This most decidedly is not the case in the plaice even during the period when the fish characteristically possesses an 'empty stomach'. In the large number of fishes I have dissected during this and other periods, the stomach is unchanged except for the contraction naturally consequent upon an empty stomach. It seems natural that Pilliet should conclude that 'l'œsophagus n'est pas, comme chez les vertébrés supérieurs, un conduit bien distinct; il contribue à former la poche stomacale', with which conclusion I must strongly disagree. But he observed that the gastric glands of the turbot did not contain parietal cells, remarking that 'on voit seulement celles qui se rapprochent du col de la glande être à la fois plus réfringentes et plus opaques que celles du fond des cul-de-sac dont le plasma est parsemé de fins granules'. Thus, the neck-cells of Gulland and the corresponding cells in the plaice did not escape his notice.

The accounts of other workers on the histology of the intestinal part of the alimentary tract do not readily lend themselves to discussion. Most investigators agree that it is extremely simple, consisting, as it does in the plaice, of a folded mucosa formed chiefly of an epithelial layer of columnar cells. These cells do not show any pronounced granular structure, nor do they indicate any differentiation except into goblet-cells. The folds to some extent simulate villi and crypts, but that these structures do not exist is shown by the absence of lacteals and granular cells such as those of Paneth. But as regards secretory power it is observed in the plaice that the nuclei of the epithelial cells are not evident in stained preparations of material fixed during a resting phase. Instead one sees a spindle-shaped mass of much greater length than the nucleus, which during digestion shows up as a small oval body, which occupies the middle of the cell and which stains heavily. In material fixed during digestion only the boundary of the nucleus and a fine chromatin network stains. That this phenomenon is connected with secretion I feel fairly certain.

Goblet-cells are not as numerous as in the oesophagus and are of a different type, possessing a filamentous basal portion

which contains the nucleus. Leucocytes are to be found between the cells of the epithelium at all times and in all zones, but are present in relatively small numbers which appear fairly constant. For this reason it appears doubtful that they play any considerable part in the absorption of food materials. There are no villi and no lacteals in the areolar tissue below the mucosa. It appears that the products of digestion pass through the epithelium into the reticulum of the areolar tissue-layer and thus directly into the blood-stream.

What has been maintained for the duodenum and intestine also holds for the pyloric caeca which are identical in histological structure with these regions, and which are in open communication with the lumen of the duodenum. It has been maintained that these are organs for storage of reserve food materials, structures concerned with secretion, with absorption, or with both these latter functions. In the plaice they are small and rudimentary or vestigial, but they present no differences in any respect from the duodenum in histological detail and are undoubtedly as much concerned with secretion and absorption as is this latter region.

Cole and Johnstone (1901) state that in the plaice 'there is no essential difference between intestine and rectum, but it is convenient to distinguish the terminal portion of the alimentary canal from that immediately preceding it'. From this passage it is clear that they overlooked the presence of an 'intestino-rectal' valve which separates the two regions. This is quite as well developed as the pyloric valve, from which it differs in deriving its musculature from the muscle-layers on each side of it and not from that on one side alone. The rectum differs from the intestine in being more abundantly supplied with goblet-cells and in having a thicker musculature. This latter may be due to a relatively greater degree of contraction following fixation. The anal sphincter is formed from the circular musculature and is much shorter than the pyloric one. It is curious that the final portion of the rectum should be devoid of goblet-cells.

ACKNOWLEDGEMENT.

I desire to express my sincere appreciation of the many kindnesses shown me by Dr. E. J. Allen, F.R.S., the Director of the Marine Biological Laboratory, Plymouth, who has spared neither encouragement nor assistance when these were needed. The work was carried out upon his suggestion at the Cawsand Laboratory of the Marine Biological Association. My thanks are also due to Dr. E. S. Russell of the Ministry of Agriculture and Fisheries, for his encouragement of the work. I have to thank also the various members of the staff at Plymouth who have assisted in any way, and the skipper and crew of the S.S. 'Salpa', who trawled the fish used and landed them at the Cawsand Laboratory, often in bad weather, during the winter of 1928.

SUMMARY.

1. Structures similar in detail to the taste-buds of mammals occur in the epithelium of the pharynx, which is considered as a distinct region for reasons given.

2. The oesophagus possesses an extremely well-developed musculature composed of striated fibres and an intensely folded mucosa liberally beset with goblet-cells. There are no glands present, and it is sharply defined from the stomach.

3. The musculature of the stomach consists of non-striated fibres, and there is no differentiation between the gastric glands at the 'cardiac' and 'pyloric' ends of the stomach, except that in the former position they are more shallow.

4. Three types of cells enter into the composition of the gastric mucosa, superficial cells forming the surface epithelium and that lining the crypts, mucus-producing cells forming the neck of the tubule, and granular cells forming the basal, secretory portion of the tubule. Parietal or oxyntic cells do not occur, nor do goblet-cells, in any part of the gastric mucosa.

5. The intestinal mucosa is folded so as to simulate crypts and villi, but it is shown that these structures do not occur. There is no differentiation of the intestinal epithelium except into goblet-cells. Leucocytes are seen in all zones of the epithelium

at all times but in relatively small numbers which appear constant, so that they probably do not play any considerable part in food absorption.

6. The pyloric caeca exhibit the same histological structure as the intestine and are in open communication with this part of the alimentary tract. They are probably secretory and absorptive like the duodenum and intestine.

7. A well-developed valve occurs at the junction of the intestine and the rectum. The rectum shows the same structure as the intestine except that the folds are deeper and more considerably beset with goblet-cells and the musculature thicker. Goblet-cells disappear from the mucosa near the anus.

LITERATURE LIST.

- (1) Agassiz, L., and Vogt, G.—“Anatomie des Salmons”, ‘Mém. Soc. sc. nat. Neuchâtel’, tom. iii, 1845.
- (2) Cole, F. J., and Johnstone, J.—“Pleuronectes”, ‘L. M. B. C. Memoirs’, 8 Dec. 1901.
- (3) Edinger, L.—“Ueber die Schleimhaut des Fischdarmes nebst Bemerkungen zur Phylogense der Drüsen des Darmrohres”, ‘Archiv für Mikrosk. Anat.’, Bd. xiii, 1877.
- (4) Greene, C. W.—“Anatomy and Histology of the Alimentary Tract of the King-Salmon”, ‘Bull. U.S. Bur. Fish’, 32, Doc. 777, 1912.
- (5) Gulland, G. L.—“The Minute Structure of the Digestive Tract of the Salmon, and the Changes which occur in it in Fresh-water”, ‘Report of Investigations on the Life-history of the Salmon in Fresh-water’, Ed. D. Noel Paton, Fishery Board for Scotland (Salmon Fisheries), 1898.
- (6) Heidenhain.—“Untersuchungen über den Bau der Labdrüsen”, ‘Arch. für Mikrosk. Anat.’, Bd. vi, 1870.
- (7) Home Everard.—‘Lectures on Comparative Anatomy’, vols. 1 et 2 avec atlas. Londres, 1814.
- (8) Macallum, A. B.—“The Alimentary Canal and Pancreas of Acipenser, Amia, and Lepidosteus”, ‘Journ. Anat. and Phys.’, 20, 1886.
- (9) Monro, H.—‘The Structure and Physiology of Fishes, explained and compared with those of Man and other Animals’. Edinburgh, 1785.
- (10) Müller, J.—“Vergleichende Anatomie der Myxinoïden, Untersuchungen über die Eingeweide der Fische”, ‘Abh. d. K. Akad. d. Wissensch. zu Berlin’, 1843.

- (11) Oppel, A.—'Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbelthiere', 1 Theil: der Magen, Iena, 1896; 2 Theil: Schlund und Darm, Iena, 1897.
- (12) Pilliet, A.—"Note sur l'estomac des Pleuronectes", 'C. R. de la Soc. de Biologie', 9^e sér., tom. x, 1893.
- (13) ——— "Recherches histologiques sur l'estomac des Poissons osseux (Pleuronectes)", 'Journ. d'Anat. et de Physiol.', tom. xxx, 1894.
- (14) Rathke, H.—'Ueber den Darmkanal der Fische', Halle, 1824.
- (15) ——— 'Bemerkungen über den Bau des Amphioxus lanceolatus'. Königsberg, 1841.
- (16) Rollett, A.—"Bemerkungen zur Kenntniss der Labdrüsen und der Magenschleimhaut", 'Untersuchungen aus d. Instit. für Physiol. und Histol. in Graz', 1871.
- (17) Stirling, W.—"On the Chemistry and Histology of the Digestive Organs of Fishes", '2nd Annual Report of the Fishery Board for Scotland', 1884.
- (18) Sullivan, M. X.—"The Physiology of the Digestive Tract of Elasmobranchs", 'Bull. of the Bureau of Fisheries', vol. xxvii, 1907.
- (19) Valatour, M.—"Recherches sur les glandes gastriques et les tuniques musculaires du tube digestif dans les Poissons osseux et les Batraciens", 'Ann. des Sc. nat.', 4^e ser., tom. xvi, 1861.
- (20) Vonk, H. J., jun.—"Le rôle des glandes de Lieberkühn en rapport avec la digestion chez les Poissons", 'Arch. néerl. de physiol.', 12, 1927.
- (21) Yung, E.—"Recherches sur la Digestion des Poissons", 'Arch. de Zool. Exper. et Gen.', 3^e ser., tom. vii, 1899.

The Gregarines of Cucumaria: *Lithocystis minchinii* Woodc. and *Lithocystis cucumariae* n.sp.

By

Helen Pixell Goodrich, M.A. (Oxon.), D.Sc. (Lond.).

With 4 Text-figures.

IN 1906 Woodcock described an interesting gregarine occurring in the respiratory trees of *Cucumaria*.¹ This parasite is neogamous and so precocious is its association that normal single vegetative forms (trophozoites) have not been seen. Apparently, as Woodcock pointed out, infection takes place by spores being drawn into the respiratory trees with currents of water through the cloacal aperture—a quite exceptional method of contamination, so far as is known. The sporozoites emerging from a ripe spore probably penetrate immediately between the epithelial cells into the connective tissue of the respiratory tree wall and there pair and round off. After absorbing food and growing at the expense of the host, they encyst. The host also forms round each pair of parasites a cyst consisting of several layers of flattened cells. These spherical cysts of various sizes are the most commonly encountered stage of the parasite, and are to be seen as conspicuous opaque white spheres through the wall of the respiratory trees on opening into the coelom. Each

¹ Woodcock recorded the gregarine from *C. pentactes* and *C. plani* (1906, p. 2), but at the time that he was working on it there was some confusion in the nomenclature of these Holothurians and they had in the year before been declared to be identical. The correct name of the former now appears to be *C. saxicola* Brady and Robertson, and that of *C. plani* is *C. normani* Pace (for specific characters see Orton, 1914). I have never found this latter species infected with any gregarine and suspect that some of Woodcock's specimens may have been wrongly identified for him at Plymouth and that possibly all the infected ones were *C. saxicola*.

cyst contains two nuclei, but generally no partition or other indication of a dual origin. They are quite soft and easily deformable, becoming much flattened when mounted under a cover-glass. These early stages have already been described by Woodcock, who recorded cysts varying from 17 to 200μ in diameter. I have been lucky enough to find still larger cysts, many containing spores, otherwise I can add little to his description. Finding nothing but vegetative stages in the respiratory trees, Woodcock was led to connect this parasite with another gregarine, *Lithocystis* (*Diplodina*, *Cystobia*) *minchinii*, which he found in the general body-cavity. This protozoan parasite was also fairly common in the *Cucumaria* that I have examined, and some further description of it is given below (p. 280).

Lithocystis cucumariae n.sp.

This seems to be the most suitable name by which to distinguish the gregarine which goes through its whole life-history in the respiratory trees of *Cucumaria*. It has appeared in only ten out of thirty-five specimens of *C. saxicola* examined from the Plymouth district since March 1927. A few of these holothurians were taken from the shallow tank in the old laboratory where they had probably been living for months. However, they seemed perfectly healthy and one individual had no infection at all, so they were not necessarily infecting each other. Another infected specimen came from Wembury Bay, and several others were taken in a trawl near the Mewstone and Stoke Point. Sometimes the infection was so heavy that the respiratory trees appeared to be more or less covered with the white cysts, while in other *Cucumaria* only two or three could be found.

Woodcock (1906) has described the vegetative stages lying inertly in the connective tissue-layer of the respiratory trees after their very early association, and his figs. 4, 18, 19, 31, and 32 represent this parasite. Many of mine have been at rather later stages than he showed in fig. 19. They were often larger—some being as much as 500μ in diameter apart from the layers

of flattened connective-tissue cells forming the host cyst. Many of the larger cysts were full of ripe spores. These cysts were 400 to 500 μ in diameter before compression and, as usual with gregarine cysts containing spores, they were rather more transparent than those containing earlier stages. They could thus be distinguished even macroscopically from the opaque cysts containing trophozoites.

The spores, crowded together indiscriminately within the cysts, were provided with long, flattened tails (Text-fig. 1) tapering to a point. They differed from most other *Lithocystis* spores in having a double funnel at the other end from the tail. The inner delicate episporal funnel was generally 2–3 μ deep.¹ When seen in profile the four upstanding edges of the two funnels had often the appearance of refringent spines, but by examining a spore from its funnel end, the delicate circular edges of inner and outer funnels could be clearly distinguished to be free from any spines or processes. While a sporozoite was forcing its way through the inner funnel it has been seen still surrounded by the outer one as usual—the use of it being quite obscure. The thick-walled endospore measured about 18 μ long and 7 μ at its widest part. The eight sporozoites were exceptionally short and broad. They were often arranged more or less in an upper and lower series round the central residuum of large refringent granules. They, however, appeared to be capable of rearranging themselves—sometimes most of them

¹ To make out the structure of the delicate episporal coat it is absolutely necessary to examine the spores fresh and it is a great help to be able to stain them. Some years ago [1915] I found that Stephen's blue-black ink was a very good stain for episporal coats and I still know of nothing better than fresh ink for staining spores mounted in water. The disadvantage of ink is the readiness with which it forms precipitates. Owing to the courtesy of one of the directors of Messrs. Stephens, one portion of the mixtures of substances incorporated in their ink and labelled by him B was tested by me and found to be ideal, since it gave no precipitate with alcohol nor any watery solution used. Unluckily I do not know the constitution of this fluid B, said not to be a simple solution. However, more recently methyl blue has also given satisfactory results especially when slightly acidified, and for permanent preparations I now use a half-saturated solution in 0.5 per cent. acetic acid and stain for some hours.

TEXT-FIG. 1.



Spore of *L. cucumariae* $\times 2,000$ approx.

were lying obliquely across the spore. Each ripe sporozoite was somewhat fusiform and about 6μ long and 2 to 3μ wide—the nucleus, consisting of a small deeply staining caryosome in a clear vesicle, being towards one end. So transparent were the sporozoites that the most conspicuous part of the spore was the central spherical residuum. The delicate tails were a little wider than the endospore proximally and tapered to a whip-like extremity, being altogether about 50μ long. The episporal processes were already formed by the time that the four-nucleated stage of the sporocyst was reached, though they were at this stage even more delicate and easily pressed out of shape, and the funnels have shown clear evidence of being formed by the folding back on itself of the episporal wall. It should be noted that this gregarine, though having a spore with a long flattened tail very like the type *Lithocystis* (*L. schneideri* Giard), is very different from it in other ways, especially in having its active vegetative stage very much curtailed owing to neogamy. No doubt its precocious association is in correlation with its habitat, for it would be obviously impossible for unattached forms to remain in the lumen of the respiratory trees owing to the currents of water passing in and out.

Occasionally a small rounded trophozoite has been found embedded in the respiratory tree wall and on examination has been found to have a single nucleus only; one of these measured as much as 150μ in diameter. In every one, however, the cytoplasm was found to be vacuolar and obviously necrotic, and there is no reason to think that solitary encystment is any more successful in this than in other gregarines.

Some early stages in nuclear division have been observed in the associates, but unfortunately no late stage and neither gametes nor zygotes have been obtained. This, no doubt, indicates that these sexual stages are passed through rapidly, as in most gregarines. A few empty cysts were found in the respiratory tree wall. There is no evidence that these cysts were burst during manipulation, so possibly the spores occasionally escape in this way and either cause auto-infection or pass to the exterior through the cloaca. We can at any rate now be

certain that this gregarine passes through its whole life-history in the respiratory trees of *C. saxicola*—a habitat from which no other parasite has ever been recorded—and the following are its specific characteristics so far known.

Lithocystis cucumariae n.sp.

Trophozoites neogamous and encyst in the wall of the respiratory tree.

Spores (approximately 18μ long) provided with an epispore produced into a double funnel at one end and at the other into a long tapering tail.

Sporozoites, short and transparent, arranged round a conspicuous residuum of refringent granules.

Habitat: respiratory trees of *C. saxicola*.

Lithocystis minchinii Woodc.

Synonyms: *Diplodina* (*Cystobia*) *minchinii* Woodcock, 1916.

Gonospora minchinii Trégouboff, 1918.

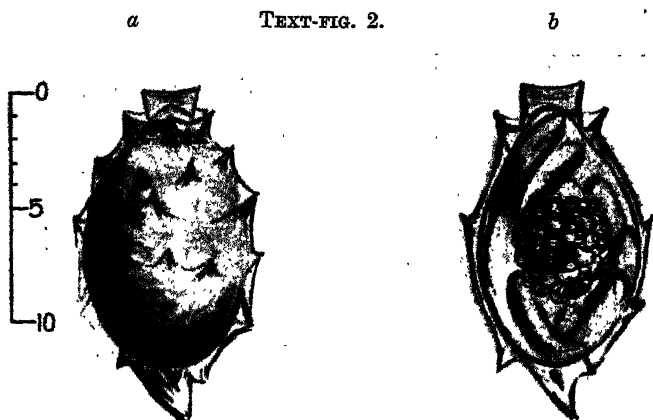
This gregarine was found parasitic in fourteen out of thirty-five specimens of *C. saxicola*, but only four times simultaneously with *L. cucumariae*. In dealing with it I should like to refer first to the spores—these being the structures of most use among gregarines for determining species.

Each spore-containing cyst was held closely to or even embedded in the coelomic wall, mesentery or a retractor muscle, being surrounded by a thick connective tissue-layer forming a host cyst. The cysts themselves were generally from half to one millimeter in diameter and full of spores—no residuum being present. When compressed, the spores broke through the tough host cyst at a central spot on the free surface (Text-figs. 3 and 4) which will be referred to below.

The spores were examined immediately, while perfectly fresh, and even then considerable difficulty was experienced in making out their very peculiar structure (footnote, p. 277).

On the shoulder of each spore (Text-figs. 2a and b), round the delicate funnel through which the sporozoites escape, the

episore is raised up into a series of conical projections forming a more or less regular outer funnel ; similar episporal projections occur at irregular intervals over the sides of the spore and at the posterior end there is a larger one forming a delicate flattened tail—to be seen whenever an exact profile view of a spore is obtained. Individual spores show much diversity in detail ; the whole episore has often, especially when fixed, the appear-



Spore of *L. minchinii* $\times 3,000$ approx. (a) Surface view ;
(b) optical section showing sporozoites and residuum.

ance of a loose thin-walled sack falling into folds about the refringent endospore.

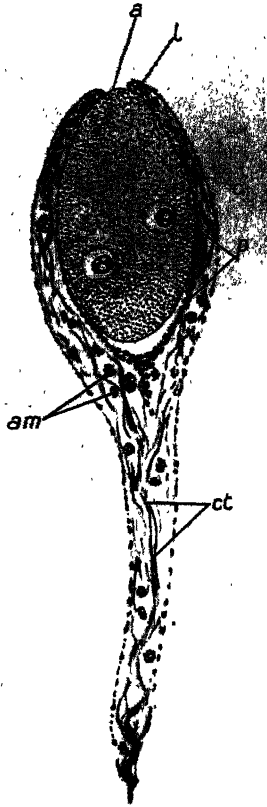
The spores are small, the endospore measuring generally 10 to 12μ by 7 to 8μ , and by far the most conspicuous part is the spherical mass of residual granules, which have a high refringency. Round this mass the eight sporozoites are variously arranged ; each is about 6μ long and almost transparent.

From their habitat, size, and general appearance, there can be no doubt that these are the spores that Woodcock¹ found and named after Professor Minchin. He said that their

¹ I am much indebted to Dr. Woodcock for putting some of his and Professor Minchin's preparations at my disposal so that I have been able to re-stain and compare them with my material.

structure was very difficult to make out, as indeed it must have been in the preserved material that he used ; some examined in

TEXT-FIG. 3.



Optical section of a pair of *L. minchinii* enclosed in cup-shaped process of the host's body-wall. For scale and lettering see fig. 4.

alcohol he described as 'crinkled and warty in outline' (1906, p. 57). Consequently, it is not surprising that the short tail was not observed and that this gregarine's affinities with *Lithocystis* were overlooked. Even now these affinities are

not very close and it may be necessary to make for it a new genus when its complete life-history is known.

In addition to cysts containing spores, I have found in the same positions in the host exactly similar cysts still containing the associated gregarines with their two nuclei unchanged or occasionally in early stages of nuclear division: these cysts were also all surrounded by thick layers of connective tissue and embedded more or less in the host's tissues, as shown in Woodcock's fig. 16. Further, there can be little doubt, I think, that the bodies shown in Woodcock's figs. 6 and 12 are, as he said, earlier stages of this same parasite. In Text-fig. 3 I have given an optical section of one of these bodies freshly detached from the body-wall of the host.

Here the associated trophozoites are enclosed in a stalked cup-shaped body, and some of the contractile fibres may be seen protruding from the proximal end of the long stalk where they have been torn away from the fibres in the connective tissue of the host's body-wall. The lips of the cup enclosing the pair of gregarines have at this stage almost met at the distal end; they do not appear to fuse so long as the cyst projects at all into the coelom (Text-fig. 4), and when compressed under a cover-glass, the cytoplasm followed by the nuclei of the gregarines could be made to shoot out with explosive suddenness through this central aperture in the host cyst. Even at the spore stage, as already mentioned, this is the only spot in the thick connective tissue cyst at which the spores could escape.

So definite were these cup-shaped bodies enclosing associated gregarines that one was tempted to suspect that they must be some special excretory organs of the host—such as the ciliated urns of *Synapta*. However, I have not been able to find them in *Cucumaria* uninfected with this gregarine, nor can I find any reference to similar structures in the literature of these Holothurians. The cup is lined with peritoneum, in places several layers thick (Text-fig. 3). The walls and stalk contain in addition to the contractile fibres many ordinary leucocytes and also a few of the large amoebocytes full of refringent globules which are of common occurrence in the general con-

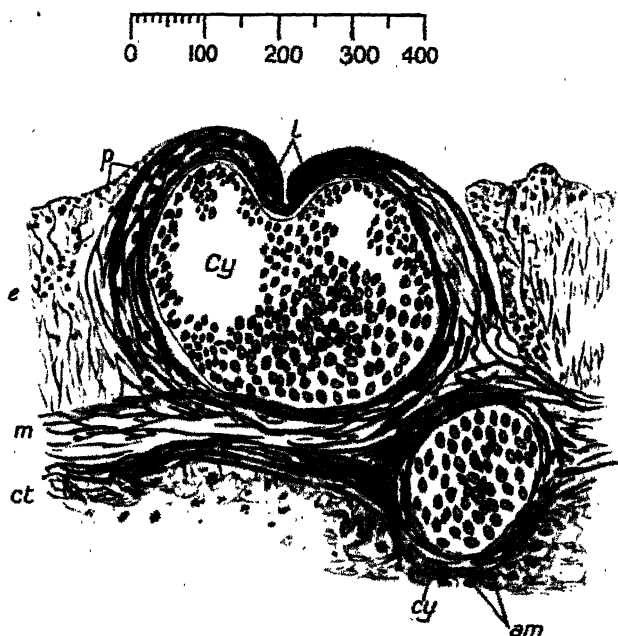
nective tissue of the body-wall. The globules of these amoebocytes stain mauve intra-vitam with cresyl blue, while the nuclei of leucocytes, peritoneal cells, and parasites stain blue.

The paired gregarines that I have measured in these cups have been from 800μ to a millimetre long. Only once have I seen any division in the cytoplasm and that was possibly an abnormal specimen, but it had a trace of a partition at its proximal end, showing, as indeed the position of the nuclei also indicates, that association has been lateral. The cytoplasm could not be made to show any sexual dimorphism by means of intra-vitam stains. The nuclei were about 42μ in diameter and appeared to contain a clear fluid in which was suspended a vacuolated caryosome of 20μ diameter. The stalks varied in length: the longest, as in Text-fig. 3, have been found on the actual body-wall where the connective tissue fibres have to pass through the circular muscles of the body-wall and then through the very thick coelomic epithelium—they were often to be found quite close to the longitudinal muscle-bands. Their relationships were confirmed by the study of serial sections through regions of the body-wall to which were attached cysts as well as the cup-shaped bodies. When once the full-grown pair of parasites has secreted its own thin and compressible cyst-wall, there can be little doubt that the stalk of the cup is shortened by the contraction of its fibres, for, although associated pairs of parasites are found in stalked cups or sessile (Text-figs. 3 and 4, and Woodcock's figs. 12 and 16), no later stages have ever been found in the stalked forms. Cysts containing spores may be seen in sections either projecting partially into the coelom beyond the epithelium or completely embedded in the body-wall, having apparently been drawn down through the muscle-layers into the connective tissue-layers. Here presumably the spores, surrounded by their very tough cyst-walls, remain imprisoned during the life of the host and thus all chance of auto-infection is prevented.

The cysts embedded thus in the thick body-wall of the *Cucumaria* are quite hidden from the outside of the animal as well as from the coelom, and were no doubt often entirely

overlooked by me at first as well as by Woodcock. This would account for the fact that they seemed to be very rare. Several hosts appeared at first to have only a single cyst and that nearly always on the large retractor muscles of the buccal region—

TEXT-FIG. 4.



Part of a section through the coelomic portion of the body-wall of *C. saxicola* showing two spore-containing cysts of *L. minchinii*. *a.*, aperture of cup; *am.*, amoebocytes; *c.*, caryosome; *ct.*, connective tissue fibres; *Cy.*, cyst protruding on to surface, median section; *cy.*, embedded cyst cut to one side; *e.*, thick layer of epithelium; *m.*, circular muscle-layer; *p.*, nuclei of peritoneum.

projecting only slightly from the surface. The thick connective tissue-wall was very tough to dissect, but when compressed the spores easily broke through the parasite cyst and emerged through the central aperture left in the host cyst or cup. On

one occasion a cyst with ripe spores was embedded in the mesentery supporting the alimentary canal, and in another *Cucumaria* I once found a cyst with the remains of a stalk free in the coelomic cavity. The contained parasites had reached an early stage of nuclear division and might possibly have developed further if they could have been removed and kept under aseptic conditions.

Strangely enough, no definite young stages of this gregarine have been seen—occasionally oval trophozoites have been found in the coelom, but they have been so limp and easily destroyed that it has often been impossible to pick them up with a pipette. They were probably specimens which had failed to pair and had become necrotic.

One may perhaps conjecture that sporozoites are liberated from spores drawn into the alimentary canal through the mouth and that they pair while finding their way through its wall into the coelom, where, if successful, they grow rapidly and stimulate the tissues of the host to grow out and envelop them, as described.

Even when they have been so enveloped they may sometimes fail to develop. Possibly if they fail to secrete a cyst the host's phagocytes are able to attack them while in these cup-shaped bodies. Anyhow, I have found occasionally that the cups contained only limp pairs such as were sometimes free in the coelom and when touched the cytoplasm easily flowed out through the aperture. Similarly empty cup-shaped bodies were sometimes found, but only in specimens of *Cucumaria* infected with this gregarine.

It will thus be seen that in order to complete the life-history of this parasite a knowledge of the early vegetative stages is necessary as well as the gametes and zygotes. These stages would no doubt be most easily obtained by artificial infection of young *Cucumaria*. Possibly the pairs enclosed in their host cup could readily be induced to continue their development *in vitro* provided they were extracted under aseptic conditions and mounted in sterile coelomic fluid. I have not been able to carry out these experiments with *Cucumaria*, but we have

already had some success in the development of gregarines from fresh-water animals 'in vitro'.

Specific characteristics of *L. minchinii* Woodcock may be given at present as follows:

1. Association lateral and neogamous.
2. The host attaches the paired parasites to its coelomic wall throughout the greater part of their lives and tries to embed them in its connective tissue.
3. Spores with peculiar episporal projections including a short flattened tail.

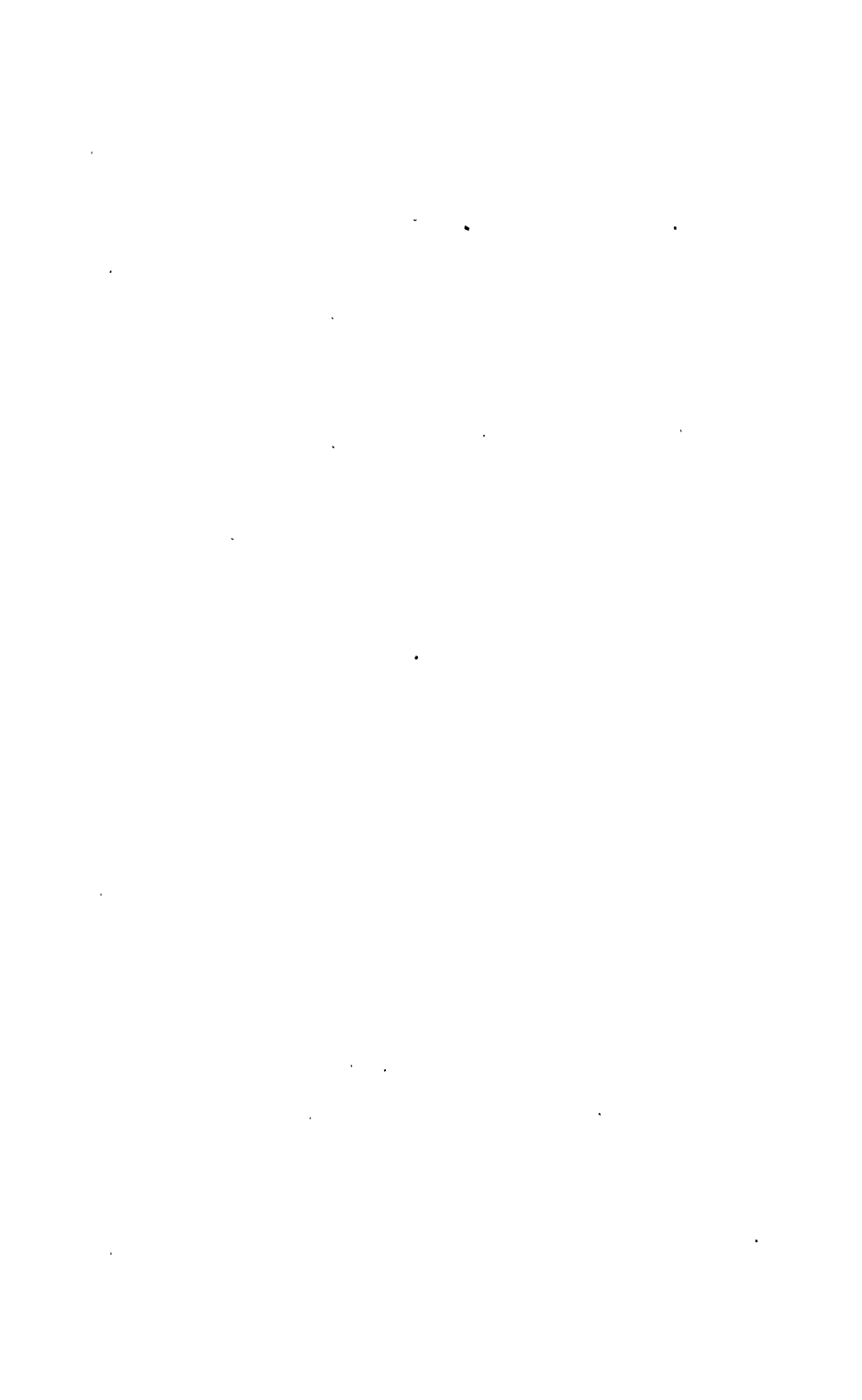
SUMMARY.

Of two neogamous gregarines infecting *C. saxicola* and hitherto considered to be one and the same species, one—*L. cucumariae* n.sp.—was restricted to the respiratory trees and had spores with long flattened tails. The other—*L. minchinii* Woodc.—was enclosed throughout most of its life in a cup-like outgrowth of the host's coelomic epithelium and connective tissue, and had spores with peculiar episporal processes including a short tail.

DEPARTMENT OF ZOOLOGY AND COMPARATIVE ANATOMY,
University Museum, Oxford.

REFERENCES.

- Goodrich, H. Pixell (1915).—"Sporozoa of Spatangoids", 'Quart. Journ. Micro. Sci.', 61, 83.
- Orton, J. H. (1914).—"On some Plymouth Holothurians", 'Journ. Mar. Biol. Assoc.', 10, 211.
- Trégouboff, G. (1918).—"Gonospora testiculi", 'Arch. Zool. Exper.', 57, 505.
- Woodcock, H. M. (1906).—"Life Cycle of *Cystobia irregularis*", 'Quart. Journ. Micro. Sci.', 50, 1.



On the Development of the Skull of *Leptodeira hotamboia*.

By

Gwendolen T. Brock, M.Sc., D.Phil. (Oxon.).

With Plates 12 and 13 and 6 Text-figs.

INTRODUCTION.

ACCOUNTS of the development of the Ophidian skull are not numerous. Rathke as early as 1839 described the development in *Coluber natrix* from a macroscopic point of view. Parker (1878) gave a full description, excellently illustrated, of *Tropidonotus natrix*, but his work is out of date and it is evident that he was misled in several points, especially over the condition of the orbito-temporal region. He does not appreciate the reduced state of the side-wall in this region, and claims to have identified both an orbito-sphenoid and an alisphenoid. Gaupp makes many valuable contributions to the understanding of the snake skull, but, as he had only a single specimen of a *Tropidonotus natrix* embryo, he was unable to settle definitely the doubtful points in Parker's work. Peyer in 1912, with the intention of bringing Parker's work up to date, gave an excellent account of the stages of development in *Vipera aspis*, but he failed to settle the problem of the orbito-temporal region satisfactorily. De Beer (1926), in his work on the orbito-temporal region, discussed the problem of the ophidian alisphenoid in the light of the present-day understanding of that bone, but had only one specimen of *Tropidonotus* and one of *Pseudechis* at his disposal. He suggested that I should undertake a full investigation of the developmental stages. The work was begun primarily in the hope of reaching a solution to the vexed problem of the 'alisphenoid'. In the course of the investigations several interesting points have come up: the

posterior attachment of the nasal capsule, the probable absence of an extracolumella and the nature of the columella attachment to the quadrate; the homology of the fenestra cochleae.

The material for study has been a series of five different stages of embryos of *Leptodeira hotamboia*. A batch of eggs, about a month old from fertilization, was given to me by the Director of the Snake Park, Port Elizabeth, South Africa. I kept them in damp earth in a temperature warm enough to force development, and took stages every four or five days. The first four stages were taken within the second month of development, and vary from $6\frac{1}{2}$ to 8 mm. in head-length. The fifth was taken after a gap of four or five weeks, a few days previous to hatching, head-length 10 mm.

Half the embryos were killed and fixed in Bouin's mixture, half in corrosive acetic mixture. The heads were stained in toto in borax carmine, sectioned, and the sections stained in piconigrosin. A blotting-paper and wax model was reconstructed from serial sections of the earliest stage.

The work has been carried out in the Department of Zoology and Comparative Anatomy, University Museum, Oxford, and I am indebted to Professor Goodrich and Mr. de Beer for their kindly advice and help, and for the loan of slides.

Basal Plate and Occipital Region.

In the model made from a four-week-old embryo of *Leptodeira hotamboia*, head-length 8 mm., illustrated in figs. 1 and 2, Pl. 12, the cartilaginous basal plate shows a dorsally concave floor suspended between the otic capsules, and extending from the foramen magnum posteriorly to the fenestra hypophyseos anteriorly. It is rectangular in shape; in an antero-posterior direction it inclines downwards in a steep curve so that the foramen magnum faces in a posteroventral direction. The anterior margin is thickened to form a crista sellaris, from the antero-lateral corners of which the trabeculae extend forward. The basal plate extends unusually far forward. When compared with *Lacerta* the distance between the facial foramen and the crista sellaris is exceptionally long. This portion in

Leptodeira forms a third of the whole plate. The basicranial fenestra, circular in outline, is situated entirely within this anterior third and therefore in front of the auditory capsules. Parker (1898) figures the same excessive length of the anterior end of the basal plate for *Tropidonotus*; Gaupp (1906) also for *Tropidonotus*, and Peyer (1912) for the viper, illustrate the same condition. It is probably universal for snakes.

The notochord extends forward in a ridge down the centre of the basal plate. In most of the specimens it does not extend as far as the basicranial fenestra. In Stage IV alone it was traced into the filling tissue of the fenestra.

The basal plate is continuous posteriorly with the occipital region. Its posterior margin is thickened to form a continuous crescentic condylar mass as described by Gaupp (1900) for *Lacerta*. Peyer's diagrams of the viper show the same structure. From the postero-lateral angles of the basal plate the occipital arches extend upwards and meet dorsally to form the tectum posterius, the whole occipital region forming a rough pentagon around the foramen magnum. The greater part of the tectum is apparently formed from the occipital region as described by Gaupp (1906, *Tropidonotus*), and Peyer (1912, *Vipera aspis*), but at its anterior end the otic capsules fuse with it, and may possibly contribute to its formation. The occipital arches are separated from the otic capsules by a posterior extension of the fissura metotica.

The foramina, through which the hypoglossal nerves pass, lie in the posterior lateral region of the basal plate. In all the specimens observed there are three foramina on each side, the most posterior lying in the occipital region. Peyer reports only two pairs of foramina in the viper. Parker (1898) says there is only one pair in *Tropidonotus*, but Gaupp (1900) and Chiarugi (1889) both find four. In Stage I of *Leptodeira* a fourth pair of foramina are present, but no nerve-roots pass through, and in the later stages they are lacking. The number of nerve-roots sometimes exceeds the number of foramina.

The vena cerebialis posterior, as in *Lacerta* (Versluys, 1896, and Gaupp, 1900), passes out of the cranial cavity

between the occipital arch and the atlas, that is, through the foramen magnum, not through the fissura metotica as in mammals.

Otic Region.

The otic region of the skull consists primarily of paired lateral auditory capsules connected ventrally by the anterior portion of the basal plate, and dorsally by the tectum posterius. But, as already pointed out, the tectum is formed almost entirely from the occipital region. In the region of the basal plate the auditory and occipital regions are confluent. An extensive fissure, the fissura metotica, separates the posterior part of the otic capsule from the basal plate. Posteriorly, the fissure bends upwards at right angles and extends between the otic capsule and the exoccipital region. Anteriorly, capsule and basal plate are fused through the basicapsular commissure. Immediately in front of the commissure is the foramen for the facial or seventh nerve, and a very slender bridge of cartilage, the pre-facial commissure, separates the foramen from the antotic incisure. Quite frequently the pre-facial commissure is lacking, and the facial foramen is without an anterior boundary. The facial foramen is situated in the angle between the anterior vestibular portion of the capsule and the cochlear prominence. The cochlear prominence is of the same proportions as in *Lacerta*, encroaching slightly on the basal plate. In front of the pre-facial commissure, the anterior margin of the otic capsule with the lateral margin of the basal plate forms the hind border of the wide incisura antotica. But this will be discussed under the orbito-temporal region.

In Stage I, the modelled stage, of *Leptodeira hotamboia* the external relief of the auditory capsule is already fairly well defined. On the lateral wall the three semicircular canals with their ampullae are recognizable as slight prominences. The anterior semicircular canal is the most prominent, being separated from the rest of the capsule by definite grooves and forming the dorsal margin of the capsule. On the medial wall the utricular prominence is well defined and the anterior and pos-

terior semicircular canals are again recognizable. There is no suggestion of a crista parotica on the lateral wall of the capsule.

The interior of the capsule is very similar to the condition described by Peyer (1912) for *Vipera aspis*, an account based on Rathke's (1839) description of *Coluber natrix*. There is a large vestibular cavity containing the utriculus and its recessus, the sacculus, and the endolymphatic duct. A ventral cavity is partly divided off from the vestibular cavity by a cartilaginous septum, the crista vestibuli, but there is a wide-open connexion between the two. The ventral cavity contains the cochlea. The lateral semicircular canal is separated from the general vestibular cavity for a short distance by a cartilaginous septum. The anterior semicircular canal lies in a cavity almost completely separated from the vestibular cavity by a strong septum. There is no septum between the anterior and lateral ampullae. Posteriorly, a ventral cavity is separated from the vestibular cavity and contains the posterior ampulla and the adjoining portion of the lateral canal.

The anterior and posterior acoustic foramina for the branches of the eighth, or auditory nerve, open on the median surface of the capsule. The anterior opening is situated on the medio-ventral aspect of the anterior portion of the cavum vestibuli, close above its separation from the cochlear prominence. The posterior opening is in the dorso-median wall of the cochlear prominence; its projecting lower lip causes the opening to face dorsally. The two foramina are completely separate even in Stage I.

The endolymphatic foramen is a small round opening, just large enough to allow the passage of the duct; it is situated some distance dorsally and posteriorly from the acoustic foramina in the median wall of the utricular prominence. At this stage there is no indication of a previous confluence with the acoustic foramina.

In the dorso-posterior aspect of the capsule there is an extensive gap in the wall of the prominence of the posterior semicircular canal. It persists without change of size up to Stage IV. In Stage V the ossified capsule shows no foramen. The aperture

in the cartilaginous capsule gives access to no nerve, blood-vessel, or duct, and is evidently merely an area of retarded chondrification.

The fenestra vestibuli in the lateral wall of the cochlear prominence is oval. The foot-plate of the columella auris almost fills it, and in early stages is indistinctly separated from the wall. In later stages the fenestra vestibuli is much larger than the foot-plate, which does not nearly fill it.

The fissura metotica has already been mentioned. It extends from the posterior edge of the basicapsular commissure backwards as a separating fissure between the basal plate and the otic capsule. At the posterior margin of the capsule, the fissure bends sharply upwards and its dorsal extension separates the otic capsule from the occipital arches. This posterior portion is very narrow and is more or less filled with tissue which in the adult is replaced by bone without deposition of cartilage. The anterior end of the fissura metotica widens out considerably and forms a distinct, though small, anterior division, known as the recessus scalae tympani (Gaupp, 1900). Medially, it is separated off from the rest of the fissure by a narrow downward projection of cartilage from the wall of the otic capsule. The projection comes in very close contact with the margin of the basal plate without actually fusing. Behind the projection the posterior division of the fissura metotica is known as the jugular foramen (Gaupp, 1900). It allows for the passage of the vagus nerve but no jugular vein passes through it. The posterior cerebral vein passes out from the cranial cavity between the basal plate and the atlas, and is joined by the vena cava lateralis to form the jugular vein. But Gaupp (1900) has pointed out that the jugular foramen rightly deserves that name in reptiles, because in very early stages a vessel, corresponding to the internal jugular vein of mammals, is present. It passes out from the cranial cavity through the jugular foramen to join the vena cava lateralis; later it atrophies, and is replaced by the posterior cerebral vein of the adult. In the earliest of my stages of *Leptodeira* the posterior cerebral is already well established, and there is no internal jugular vein.

According to Gaupp (1900), the usual course for the glossopharyngeus nerve is through the recessus scalae tympani in reptiles, not through the jugular foramen as in mammals. In *Leptodeira hotamboia* it penetrates the cartilage of the basal plate immediately below the median aperture of the recessus scalae tympani. It does not actually enter the recessus but passes through a channel in the cartilage of the basal plate below it. Its exit to the exterior is in close proximity to the jugular foramen. This nerve merges into the vagus ganglion, from which a branch passes forward to the pharynx and tongue, and this leaves little doubt that it is the glossopharyngeus.

But Peyer (1912) reports that the glossopharyngeus nerve in *Vipera aspis* actually passes through the posterior part of the fissura metotica, the jugular foramen. He also describes an 'undetermined' nerve which passes through the apertura medialis of the recessus scalae tympani, into the cochlear capsule, and out through the apertura lateralis. Rice (1920) suggests that the 'undetermined' nerve is the true glossopharyngeus and that Peyer has mistaken a branch of the vagus for the glossopharyngeus. Möller (1905) reports an intracapsular course for the glossopharyngeus in *Vipera aspis*. According to Rice's summary (p. 152) an extracapsular course is much more general, the turtles being the only reptiles which regularly show an intracapsular course. I can find no nerve in *Leptodeira* corresponding to Peyer's 'undetermined' nerve or Möller's glossopharyngeus. The course is the normal reptilian one, through the recessus scalae tympani, the only variation being that the margin of the basal plate has surrounded it. It is conceivable that in *Vipera aspis* the nerve has been surrounded by the capsular wall instead of by the basal plate, thus bringing about an intracapsular position.

The recessus scalae tympani is situated immediately behind the cochlear prominence. An aperture in the posterior floor of the cochlear capsule faces into the recessus. It is the fenestra cochleae and is situated directly below the fenestra vestibuli, a narrow strip of cartilage separating the two openings.

In transverse section (fig. 21, Pl. 13) the recessus scalae

tympani appears triangular, the three points of the triangle being the lateral and medial walls of the auditory capsule and the edge of the basal plate. There is a median aperture from the recessus to the cranial cavity, and a lateral aperture to the exterior, corresponding exactly to Gaupp's figure (1900) of *Lacerta*. The recessus scalae tympani, then, forms a series of three communicating spaces :

- (1) from the otic cavity to the exterior, through the fenestra cochleae and lateral aperture ;
- (2) from the otic cavity to the cranial cavity, through the fenestra cochleae and medial aperture ;
- (3) from the cranial cavity to the exterior, through the medial and lateral apertures.

In early stages the recessus scalae tympani is filled with a loose embryonic tissue in which are irregular gaps ; these later coalesce to form the perilymphatic sack. The perilymphatic duct of the labyrinth cavity leads out through the fenestra cochleae into the recessus scalae tympani, where it expands into the perilymphatic sack. It then passes through the medial aperture of the recessus and communicates with the sub-arachnoidal lymph-spaces of the cranial cavity. In later stages this communication is lost. The perilymphatic sack entirely fills the recessus, and presses outwards through the lateral aperture against the rudimentary tympanic cavity. The bounding wall of the perilymphatic sack, where it fills the lateral aperture, with the bounding wall of the tympanic cavity together represent the membrana tympani secundaria of *Lacerta* (Gaupp, 1900), which closes the aperture lateralis of the recessus scalae tympani. But in *Leptodeira* it scarcely deserves that name. The bounding wall of the rudimentary tympanic cavity is not very strong and it does not combine with the wall of the perilymphatic sack and intermediate tissue to form the stout membrane of *Lacerta*.

The relationship between the fenestra cochleae of reptiles and the fenestra cochleae or rotunda of mammals is an interesting problem. Gaupp, in 1900, disagreeing with the earlier work of Versluys (1899), put forward the conjecture that the apertures

in mammal and reptiles were homologous. Admittedly, there are differences in the condition of *Lacerta* and man. In the latter the opening faces laterally, in the lizard ventrally. In man it faces towards the tympanic cavity with the membrana tympani secundaria stretched across it; while in the lizard the opening is from the otic cavity into the recessus scalae tympani, and has no membrane stretched across it, the membrana tympani secundaria being stretched across the apertura lateralis of the recessus. In man the perilymphatic spaces are entirely within the otic cavity; but in the lizard the perilymphatic sack protrudes through the fenestra cochleae into the recessus scalae tympani.

Gaupp supposes that the mammalian condition is brought about by the division of the primary fenestra cochleae of the reptile, or foramen perilymphaticum as he terms it, into the definitive foramen rotundum and cochlear aqueduct of the mammal.

He says: 'In Bezug auf die Lacertilier kann aber wohl als sicher gelten, dass das in der Ohrkapsel befindliche, in den Recessus scalae tympani führende grosse Foramen, aus dem der Saccus perilymphaticus heraustritt, ganz oder doch in der Hauptsache der Fenestra cochleae s. rotunda der Säuger entspricht, und es ist nur eine Einschränkung, die möglicherweise notwendig sein wird, nämlich die, dass sich vielleicht von ihm auch die als aquaeductus cochleae bezeichnete Öffnung ableitet' (1900, p. 515).

In accordance with this theory he believes that the membrana tympani secundaria of the mammal is only physiologically, not morphologically, homologous with the membrane of the same name in the lizard. It lies across quite a different aperture, and has an entirely capsular rim instead of being stretched from capsule wall to basal plate.

Gaupp (1902) demonstrated a primitive reptilian condition in *Echidna*, and believed that further investigation of mammalian skull ontogeny would show the division of the primary foramen perilymphaticum into fenestra rotunda and cochlear aqueduct. E. Fischer (1903) demonstrated in an embryo *Semnopithecus* a dividing process springing from the

anterior margin of the foramen perilymphaticum. Voit (1909) for the rabbit, Olmstead (1911) for the dog, Macklin (1914) and Kernan (1916) for the human embryo, and Terry (1917) for the cat, all demonstrate intermediate conditions in the division of the primary foramen perilymphaticum. Voit names the process the 'processus intraperilymphaticus'.

I have been enabled to examine sections of a cat embryo which are very similar to those illustrated by Terry (1917), and also sections of a mouse embryo, a ferret, and a hedgehog, and I do not agree with Gaupp's interpretation.

I believe that the fenestra rotunda of the mammal corresponds not to a portion of the primary fenestra cochleae, or foramen perilymphaticum, but, more or less closely, to the apertura lateralis of the recessus scalae tympani in reptiles.

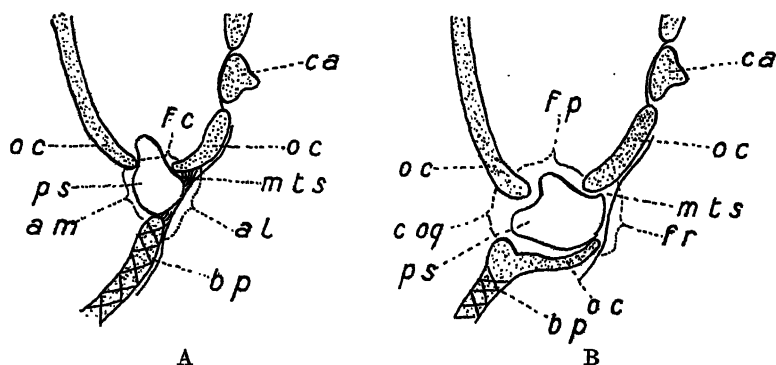
Text-fig. 1 A and B give a diagrammatic comparison of the condition in the reptile and mammal. They represent transverse sections through the region of the fenestra cochleae and fenestra rotunda. In A (reptile) the perilymphatic sack is seen protruding from the labyrinth cavity, through the fenestra cochleae, into the recessus scalae tympani. The bounding wall of the sack makes a circular sweep from the rim of the fenestra cochleae; stretching down to the margin of the basal plate, it forms a membrane round the whole recessus scalae tympani which closes both lateral and medial apertures of the recessus. Its lateral extent from the margin of the fenestra cochleae to the basal plate comes in contact with the wall of the tympanic cavity and these two membranes, with the intermediate tissue, form a stout membrani tympani secundaria.

In B (mammal) the perilymphatic sack lies within the cochlear capsule. It protrudes slightly through a lateral aperture in the wall of the cochlear capsule, the fenestra rotunda, pressing against the wall of the tympanic cavity and forming with it the membrana tympani secundaria. The membrane is stretched from capsular wall to capsular wall, not from capsular wall to basal plate as in the reptile (A).

Nevertheless, I consider that the fenestra rotunda in B corresponds to the apertura lateralis in A, the membrana tympani

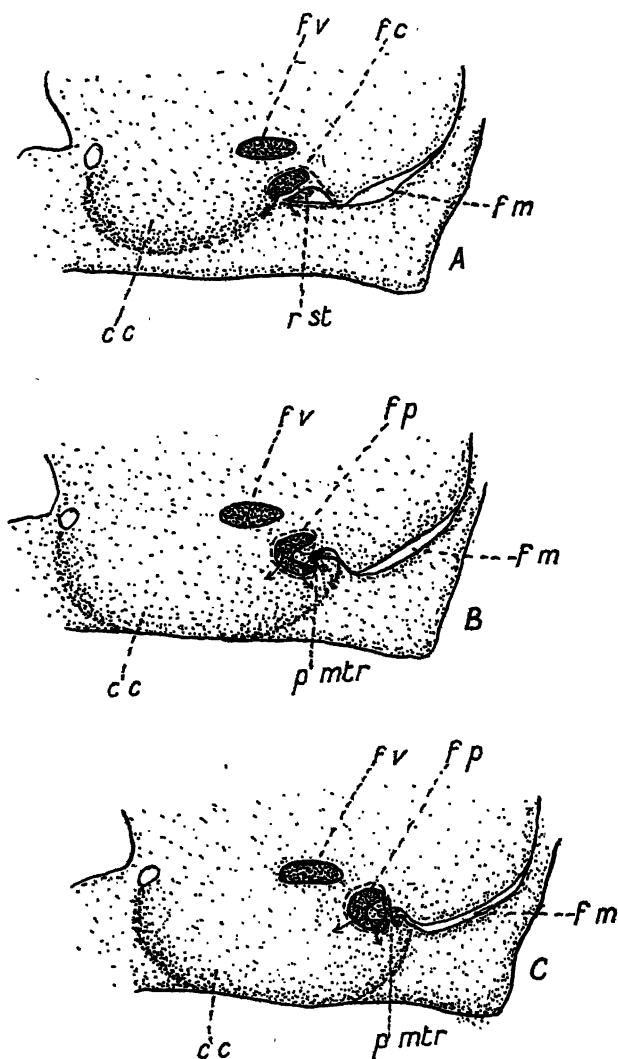
secundaria in both being homologous. I believe that the portion of the cochlear capsule enclosing the perilymphatic sack represents the recessus scalae tympani of the reptile which has become included within the capsule.

In a model of an embryo cat, lent by Mr. de Beer, of which Text-fig. 2 B (see next page) is a diagrammatic partial representation, the fenestra rotunda faces laterally, and the plane



TEXT-FIG. 1.

of the membrana tympani secundaria is lateral. The primary foramen perilymphaticum faces caudally; its lateral margin is in the plane of the apertura lateralis and membrana tympani secundaria, but its medial margin is situated in a more internal plane, and its aperture opens into a reduced intracapsular recessus scalae tympani. The rudimentary processus intraperilymphaticus, it is true, suggests a division of the foramen perilymphaticum into fenestra rotunda and cochlear aqueduct, as Gaupp surmised, but only in the same sense that the margin of the basal plate in the reptile separates the apertura lateralis from the apertura medialis. In those mammals, the dog (Voit) and *Semnopithecus* (E. Fischer), in which the processus intraperilymphaticus is completed, it must close off the fenestra rotunda from the jugular foramen (Text-fig. 2 C, and Text-fig. 3), forming a posterior support to the membrana tympani secundaria. In this sense, that is, in function, it corresponds to the



TEXT-FIG. 2.

separating bar between the recessus scalae tympani and jugular foramen of the reptile, but the two structures cannot be regarded as morphologically similar.

This transformation from the reptilian to the mammalian condition can readily be understood if we suppose that the very much enlarged cochlear duct of the mammal causes the enlarging cochlear capsule to encroach backwards at the expense of the basal plate. Text-figs. 2 A, B, and C represent such a transformation. In A (reptile) the fenestra cochleae faces into the recessus scalae tympani. The arrows indicate the three communicating passages of the recessus (1) from the otic capsule to the exterior, (2) from the otic cavity to the cranial cavity, and (3) from the cranial cavity to the exterior.

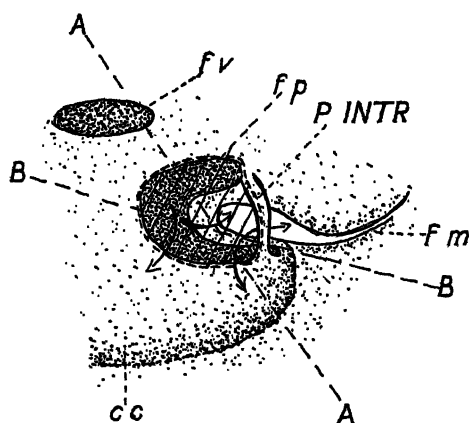
The area surrounded by the dotted line represents the membrana tympani secundaria.

The ventral posterior region of the cochlear capsule expands in a posterior direction carrying with it the ventral end of the fenestra cochleae, or foramen perilymphaticum, which is drawn into a semicircular shape (Text-fig. 2 B).

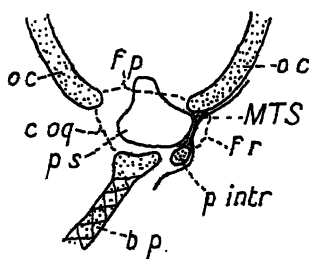
In C the posterior enlargement of the cochlear capsule comes almost in contact with the wall of the vestibular cavity. The margin of the basal plate has been pressed back and the recessus scalae tympani is surrounded by capsule and becomes largely included within the labyrinth cavity. A projection of cartilage, the processus intraperilymphaticus, divides the fenestra rotunda from the jugular foramen and the membrana tympani secundaria is stretched across the lateral aperture. The foramen perilymphaticum, facing caudally, would be the homologue of the fenestra cochleae of the reptile.

Text-fig. 3 is an enlargement of Text-fig. 2 C, to show the relations of the fenestra perilymphaticum, fenestra rotunda, and processus intraperilymphaticus. The dotted lines A-A and B-B show the planes through which the sectional diagrams, Text-fig. 1 B, and Text-fig. 4, are taken. Text-fig. 1 B, as already described, is a transverse section through the region of the foramen perilymphaticum. Text-fig. 4 is taken in a more horizontal plane and passes through the processus perilymphaticum. Text-fig. 4 corresponds very closely with Terry's (1917) figure of the cat and also with Fischer's (1908) figure of *Semnopithecus*.

Although the cochlear aqueduct of the mammal is separated from the fenestra rotunda by the processus intraperilymphaticus, it is not separated from the jugular foramen, as shown



TEXT-FIG. 3.



TEXT-FIG. 4.

in C. The separating bar between the recessus scalae tympani and jugular foramen of the reptile may have been lost. More probably the backward expansion of the cochlear capsule has obliterated the original anterior division of the fissura metotica, and the mammalian cochlear aqueduct represents the anterior limit of the original jugular foramen. Whichever be the explanation, it makes little difference to the present argument. The recessus scalae tympani of the reptile, I take it, is that part

of the fissura metotica which is occupied by the perilymphatic sack. If the enlargement of the cochlear capsule in the mammal does carry it some distance caudally along the original extent of the fissure, it still represents the recessus scalae tympani. Through its lateral aperture the bounding wall of the perilymphatic sack still comes in contact with the tympanic cavity-wall to form the membrana tympani secundaria.

With reference to this membrane Rice (1920) makes a similar statement: 'I believe that the lateral part of the membrane filling the fenestra cochleae of stage 6 of *Eumeces* (corresponding to the filling of the lateral aperture of the recessus scalae tympani in *Lacerta* and stage 5 of *Eumeces*) may be safely homologised with the secondary tympanic membrane of the mammal, while the median portion (corresponding to the filling of the median aperture) occupies the position of the aqueductus cochleae of the mammal' (p. 152).

I have taken the description of the typical reptile from Gaupp's description of *Lacerta* (1900). At this point it would be well to consider how closely the other reptilian classes conform to this typical condition. It has already been shown that *Leptodeira* conforms very closely. *Crocodylus* at first sight appears rather different. The fenestra cochleae faces laterally instead of ventrally. The margin of the basal plate has grown up dorsolaterally in a processus basicapsularis which partially covers the aperture (Shiino, 1914). A stout membrana tympani secundaria is stretched from the upper edge of the fenestra cochleae to the edge of the processus basicapsularis; that is, from capsular wall to basal plate. In this *Crocodylus* is essentially reptilian; the aperture closed by the membrane is similar to the apertura lateralis of *Lacerta*, and the fenestra cochleae is an opening into the recessus scalae tympani.

Chrysemys marginata shows an interesting variation from the typical condition. The cochlea is larger than in *Lacerta* and has encroached backwards slightly at the expense of the basal plate. In this way the fenestra cochleae has assumed a vertical position, facing posteriorly, or caudally, into the recessus scalae tympani, and the floor of the recessus is

partly capsular. It would appear that the condition in the turtle is intermediate between the typical reptilian and mammalian conditions.

To summarize this discussion, I consider that the fenestra cochleae of the reptile, through which the perilymphatic duct makes its exit from the labyrinth cavity, does not correspond to the fenestra rotunda of the mammal. The fenestra rotunda corresponds to the apertura lateralis of reptiles. The membrana tympani secundaria corresponds morphologically as well as functionally with that of the mammal. The fact that the membrane stretches from capsule to capsule in the mammal, while in the reptile it is from basal plate to capsule, may be accounted for by the backward encroachment of the enlarging cochlear capsule at the expense of the basal plate.

These conclusions are somewhat similar to those of Versluys (1899).

He says: ' In ihrer Function und der Hauptsache nach auch in der Lage am Schädel entspricht diese Membran demnach der Membrana tympani secundaria der Mammalia, das Foramen jugulare aber der Fenestra rotunda ' (p. 358).

Versluys's investigations were on the fenestra rotunda of the bird, and he compares the condition in bird and reptile. The goose he finds differs very little from the reptile, but for the fowl he describes a condition very similar to that I have described for the typical mammal.

' Der Recessus scalae tympani des Huhns ist demnach ein abgetrenntes Stück des Jugularis-Canals, die Fenestra rotunda ein Theil des Foramen jugulare externum. Dagegen entspricht der Recessus scalae tympani der Lacertilia dem ganzen Jugularis-Canal, sein äusseres hoch vollständig dem Foramen jugulare externum. . . . Es (Fenestra rotunda des Huhns) ist jedoch bestimmt nicht das Loch, durch das der Ductus perilymphaticus aus der Labyrinthhöhle in den Recessus tritt ' (p. 356).

Versluys's jugular canal corresponds to the recessus scalae tympani of Gaupp's terminology. Versluys believed that in the primary reptilian condition the jugular vein passed through the

anterior division of the fissura metotica, the same section which contained the perilymphatic sack. He called this section the jugular canal, and its lateral aperture foramen jugulare externum, Gaupp's apertura lateralis of the recessus scalae tympani.

Thus Versluys's conclusions on the relation between fowl and reptile are that the fenestra rotunda of the fowl does not correspond to the fenestra cochleae of the reptile but to a portion of the apertura lateralis of the recessus scalae tympani.

The Columella Auris.

The columella auris is a slender rod of cartilage ; its oval footplate is inserted in the fenestra vestibuli of the cochlear capsule. The shaft extends outwards and slightly downwards from the side of the otic capsule. Its distal end bends sharply from the axis of the proximal end, and continues in a posterior and ventral direction. It comes in close contact with the posterior median surface of the quadrate, but may be distinguished by its procartilaginous condition when the quadrate is already well-defined cartilage. At a later stage a small nodule of cartilage differentiates from the distal end of the uniform procartilaginous rod and fuses with a projection from the quadrate. This is undoubtedly Parker's stylohyale (1878). In the adult there is an articulating joint between the end of the columella and the stylohyale.

With the absence of a tympanic membrane no insertion plate is present, and no recognizable extracolumella. But there is a wide divergence of opinion in the literature on the ophidian columella auris as to the nature of its component parts. Gadow regarded Parker's stylohyale as the extracolumella. Rice (1920) makes the tentative suggestion that it is in the nature of a connexion from the processus accessorius anterior of the insertion plate, similar to that described by Fuchs (1909) for *Lacerta* ; this would be to regard the distal end of the columella rod as extracolumella. Okajima (1915) found that the extracolumella was entirely lacking, ontogenetically and morphologically, in *Trigonocephalus*. He states that the stylohyale is essentially a process of the quadrate, having nothing to do primarily

with the columella auris. But from my observations on *Leptodeira hotamboia* and on a very young night-adder embryo, I am inclined to agree with Möller (1905, on *Vipera aspis*) and conclude that the nodule originates from the columella auris and secondarily fuses with a projection of the quadrate. Peyer (1912) agrees that the stylohyale in *Vipera aspis* is a part of the columella auris in origin, and possibly an extracolumella.

The relations of the nerves and blood-vessels to the distal bent end of the columella in *Leptodeira hotamboia* have an important bearing upon the problem of the extracolumella. The vena capitis lateralis passes forward over the shaft of the columella auris and lies between the bent distal end and the wall of the otic capsule. The orbital artery is given off from the internal carotid artery some distance anterior to the columella. This is an interesting variation from the usual course in Reptilia, which is up and over the shaft from an origin posterior to the columella. But another unusual course is found in *Sphenodon* (Versluys, 1903, and Wyeth, 1924), where the orbital artery passes beneath the columella, and in *Hemidactylus* and other Geckones (Versluys, 1903) the orbital artery pierces the shaft, as it does in *Gymnophiona* and also in the mammals.

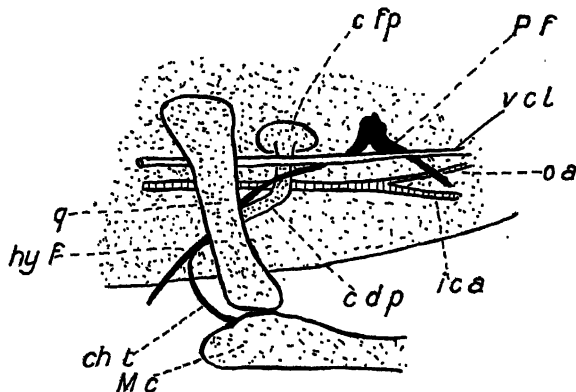
The hyomandibular branch of the facial nerve passes back over the shaft of the columella, then bends outwards and downwards below the bent distal end. The chorda tympani is given off below and posterior to the columella extremity, and runs forwards and downwards to the median side of Meckel's cartilage. Text-fig. 5 shows the relation of the nerves and blood-vessels to the columella.

It is a well-established fact that the course of the chorda tympani in all amniotes is exceedingly uniform, and is an important factor in determining the homologies of the various parts of the ear bones. The hyomandibular branch of the facial always passes backwards over the shaft of the columella medial to the dorsal and internal processes. The chorda tympani is given off posterior to the columella, loops round the dorsal

process and passes back over the insertion plate of the extracolumella (see text-fig. 2, Goodrich, 1916).

Since in *Leptodeira hotamboia* the chorda tympani passes under the distal bent end of the columella, it cannot be an extracolumella, and therefore should not be called a stylohyale.

A further fact weighing against the recognition of an extra-



TEXT-FIG. 5.

columella is the complete ossification of the whole columella structure in *Leptodeira*. It is generally accepted that the medial part of the columella, the otostapes, ossifies, but the distal portion, the extracolumella or hyostapes, remains cartilaginous. Okajima found that the lateral third in *Trigonocephalus*, the part he regards as of quadrate origin, remained cartilaginous, but Möller and Peyer both found that the whole structure ossified in *Vipera aspis*, though Peyer says the stylohyale ossifies much later than the rest.

Goodrich (1916) pointed out that the recesses of the tympanic diverticulum bear a constant relation to the columellar elements. The tympanic diverticulum of *Lacerta* has three dorsal recesses: (1) an anterior median which bends from in front over the columella shaft and is median to the dorsal and internal processes; (2) an anterior lateral; and (3) a posterior lateral.

These last two are lateral to the dorsal and internal processes; they are respectively anterior and posterior to the extracolumella shaft, and meet above it, thus encircling the shaft of the extracolumella in a ring-like diverticulum.

In all snakes the tympanic cavity is imperfectly developed and no tympanic membrane is formed. In all the stages of *Leptodeira* an ill-defined tympanic diverticulum is present, which extends up round the columella auris. Even the first stage, however, is too old to show the diverticulum recesses which might help in the identification of the distal process (fig. 21, Pl. 13).

It seems fairly evident, however, that the backwardly bent distal end of the columella corresponds either to a processus dorsalis or a processus internus. It lies in the loop of the chorda tympani and hyomandibular nerve and it is lateral to the vena capitis lateralis. If it be regarded as homologous with the dorsal process of *Lacerta*, then the nodule, Parker's stylohyale, would represent the intercalare. It is conceivable that, with the backward migration of the quadrate to facilitate the wide gape and with the absence of a processus paroticus, the intercalare loses its connexion with the otic capsule. This is paralleled in crocodiles where the dorsal process is not connected with the otic capsule, but fuses with the quadrate in the region of the otic process. Unlike crocodiles, the nodule of *Leptodeira* fuses with the mid-region of the quadrate, not with its most dorsal margin, where one might expect to find the otic process. This might be accounted for by the elongation and backward rotation of the quadrate. In contrast to *Lacerta*, this intercalare would be in connexion with the columella auris through the persistent dorsal process, but this is also the case in crocodiles (Goldby, 1925) and in *Sphenodon* (Versluys, 1908, and Wyeth, 1924).

If the process be regarded as homologous with the processus internus of *Lacerta*, there is no possible explanation of Parker's stylohyale. The relations of the nerves and blood-vessels and tympanic diverticula would be the same to the internal process as to the dorsal process, if the internal process

attached to the quadrate were swung over with the backward migration of the quadrate. The internal process in Reptilia is of much less general occurrence than the dorsal process, and in *Lacerta* appears much later in development. The dorsal process is possibly the more primitive structure, and I incline to homologize the distal bent end of the columella in the snake and its stylohyale with the dorsal process and intercalare of other reptiles.

Orbito-temporal Region.

The condition of the orbito-temporal region in snakes generally is very uniform. The cartilaginous skeleton is to a large extent lacking. The trabeculae of the basis cranii in *Leptodeira* surround the fenestra hypophyseos. They meet in front and extend forward a long way parallel to one another without fusing. There is no trace of an interorbital septum, either cartilaginous or membranous. Peyer (1912) mentions that an interorbital septum is present in *Tropidonotus* as thickened tissue, but does not detect any trace of it in the viper.

The cartilaginous side-wall in the anterior orbital region is entirely lacking. I find no trace of the orbito-sphenoid cartilage described by Parker (1898) for *Tropidonotus*. Peyer fails to find an orbito-sphenoid cartilage in the viper, and disagrees with Parker's account of *Tropidonotus*. The side-wall is replaced entirely by membrane which in later stages is invaded by descending processes of the parietals and frontals.

In the temporal region, also, the side-wall is lacking. A prefacial commissure may be present or the facial foramen may be confluent with the trigeminal incisure. There are no pilae antoticae or taenia marginales and the trigeminal nerve passes out through a widely open trigeminal incisure. In the lateral margin of the basal plate, ventral to the trigeminal incisure, and posterior to the crista sellaris, is an extensive gap in the cartilage of the plate. It is closed by membrane, and no nerves or blood-vessels pass through it. Gaupp (1902) describes a similar gap in the lateral margin of the basal plate in *Tropidonotus*, and de Beer (1926) figures the same for *Pseudechis*. Its

significance is unknown; it is apparently just a part of the general reduction of the cartilaginous wall of the snake-skull. As in the anterior region, the lack of cartilaginous wall is compensated by the development of a thickened tissue membrane.

From the margin of the basal plate in the region of the gap described above, a short blunt process projects outwards and slightly upwards. It projects into the thickened tissue which extends upwards to the lateral wall of the otic capsule. In this way the trigeminal ganglion is enclosed with the head vein in a cavity which is bounded mesially by the dura mater and laterally by membrane stretching from the lateral process of the basal plate, or trabecular plate it may be called in this region, to the lateral wall of the otic capsule.

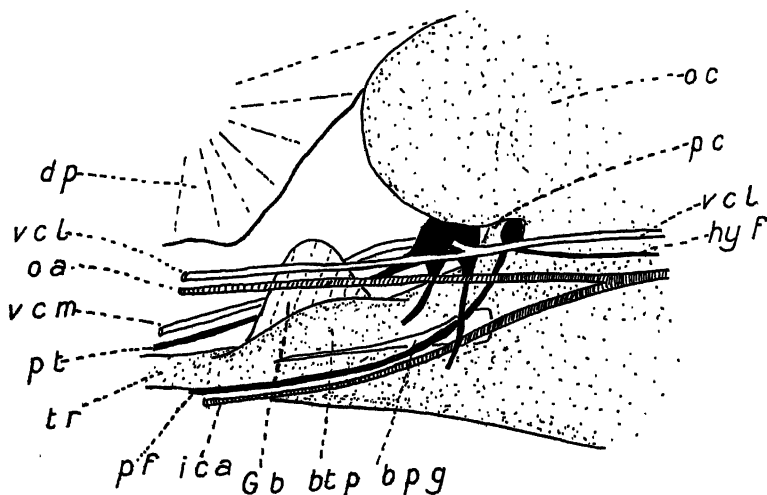
In Stage IV of *Leptodeira*, shown in fig. 10, Pl. 13, a splinter of bone extends upwards from the edge of the cartilaginous projection of the trabecular plate into this membrane. (I hope to show that the cartilaginous process is a basitrabecular process (Goodrich) or basipterygoid process (according to previous usage); for convenience, therefore, I shall call it a basitrabecular process.)

Fig. 11, Pl. 13, shows the margin of the trabecular plate, posterior to the basitrabecular process, extending upwards in close contact with the dura mater and medial to the membrane and its bony column. A few sections further back it is continuous with the prefacial commissure. The membrane in the region of the trigeminal incisure is, therefore, though continuous with the membrane of the orbital region, definitely lateral to the line of the original skull-wall as shown by the prefacial commissure and trabecular plate margin. In Stage IV there is a noticeable gap between the bone of the descending process of the parietal and the bony column on the basitrabecular process.

In Stage V (fig. 15, Pl. 13), a month later, the whole membrane has ossified. It extends to the wall of the otic capsule, overlapping the pro-otic bone, and covering the facial foramen. Its anterior edge is in contact with the descending process of the parietal. It is lateral to and covers the profundus nerve and trigeminal ganglion. The maxillary and mandibular branches

of the trigeminal both pass out through the bone, each having a separate foramen.

Gaupp (1902) described a similar bone, separating the maxillary and mandibular branches of the trigeminal nerve, in an adult *Tropidonotus* and adult *Dipsadomorphus*, and Hallman (1887) found it in the adult *Python*. For convenience I shall refer to the bone as Gaupp's bone.



TEXT-FIG. 6.

Peyer (1912) mentions a bone in this region, but regards it as an extension of the pro-otic bone. This will be discussed later.

Text-fig. 6 gives the relations of the chief nerves and blood-vessels to the basitrabecular process and to Gaupp's bone in its early stages (Stage IV of *Leptodeira hotamboia*). The mandibular and maxillary branches of the trigeminal nerve pass out over the margin of the trabecular plate posterior to the basitrabecular process. They pierce through the membrane which bounds the incisura antotica laterally. The profundus branch does not pass out through the incisura antotica. It is not included in the trigeminal ganglion, and does not even pierce the dura mater immediately, but runs forward for a short dis-

tance actually within the cranial cavity. Its position is median to the basitrabecular plate and Gaupp's bone. When it penetrates the dura mater it comes to lie in a space between the dura mater and the descending process of the parietal. It swells out into an ophthalmic ganglion.

The vena capitis lateralis passes forward over the shaft of the columella auris and continues forward lateral to Gaupp's bone. It gives off a branch which passes through the membrane of the trigeminal incisure into the space between it and the dura mater where it joins the vena capitis medialis. The vena capitis medialis passes forward in company with the profundus in the space between the dura mater and the parietal descending process. It gives off a small pituitary branch, anterior to the crista sellaris which joins its fellow of the other side. In Stage V the vena capitis medialis has disappeared. The course of the vena capitis lateralis is entirely outside the skull-wall, figs. 12 and 13, Pl. 13.

The internal carotid artery has the usual course lateral to the side-wall of the skull. It passes under the basitrabecular process, and enters the cranial cavity from a ventral direction, through a notch in the posterolateral angle of the fenestra hypophyseos, not through a separate foramen. As already mentioned, the orbital artery has its origin unusually far forward, that is, in front of the columella auris. It passes forward lateral to Gaupp's bone.

The facial nerve emerges through the facial foramen, or, when a prefacial commissure is lacking, through the same incisure as the trigeminal nerve. Its posterior branch, the hyomandibular, has already been described in connexion with the columella auris. The palatine branch runs down and forwards beneath the basitrabecular process. It accompanies the internal carotid artery in its course outside the skull-wall. It lies immediately dorsal to it and within a groove in the ventral surface of the basal plate. In Stage V the parasphenoid bone has covered them and forms the outer wall of a parabasal canal enclosing the nerve and artery.

The abducens nerve originates immediately below the trige-

minal. It has a special canal excavated in the dorsal surface of the basal plate lateral to the crista sellaris (fig. 13, Pl. 13). The anterior opening of the canal is still within the cranial cavity. The nerve bends upwards from the opening of the canal and enters the space between the dura mater and the descending process of the parietal. It passes forward lateral to the profundus nerve, buried in the thickened tissue of the lateral wall of the space, and leaves the skull cavity round the anterior margin of the descending process of the parietal in company with the profundus. Gaupp (1902, *Tropidonotus*) and Peyer (1912) for the viper describe similar courses for the abducens nerve, but Gaupp regards the intracranial exit of the abducens canal as peculiar to snakes and as a result of the downgrowth of the parietal. Rice (1920), working on *Eumeces* and *Lacerta*, concludes that it is typical of reptiles. In *Eumeces* the abducens canal has its anterior opening in the cartilage of the basal plate within the cranial cavity, and the nerve has its exit through the foramen metopticum. This is very closely in line with the condition described for *Leptodeira*; the exit through the foramen metopticum in the lacertilian would correspond to the entrance of the nerve in the snake into the intermediate space bounded by the parietal descending process. The presence of this bony descending process necessitates that the final exit of the abducens nerve be through the foramen orbitale magnum (Gaupp, 1902). The trochlear and oculomotor nerves also pass forward in this intermediate space between dura mater and descending process and have their exit from the skull through the foramen orbitale magnum with the optic nerve and the abducens.

Gaupp (1902) described this intermediate space between the dura mater and the descending process of the parietal in his account of *Tropidonotus*. He pointed out that in the lizard the profundus, the abducens, the trochlear, oculomotor, and optic nerves pass through various separate foramina in the side-wall of the cartilaginous cranium, while in the snake the descending process of the parietal is equivalent to an additional wall outside the original cranial wall. It encloses the five nerves

in a common passage. The opening between the parietal and frontal bones through which the three eye-muscle nerves and the optic nerve make their exit, he has named the foramen orbitale magnum, and it does not correspond to the optic foramen in the lizard, which is an opening in the cartilaginous wall through which the optic nerve alone passes.

In the snake the original cartilaginous cranial wall has completely disappeared, leaving this intermediate space between the parietal and the dura mater. Gaupp regards the space as extracranial, something outside the original cranial wall, the perichondrium of which must have been in close contact with the dura mater on the medial side of the intermediate space. De Beer (1926) thinks that the space should rather be regarded as intramural, lying between the dura mater and the original cranial wall which must have been close inside the existing parietal wall. He points out that the dura mater is a long way internal to the trabeculae, the basal plate and the otic capsule which are in line with the dense mesenchyme foreshadowing the parietal downgrowth. The condition in *Leptodeira hotamboia* confirms de Beer's conclusion that the space is intramural and the result of inward shrinkage of the dura mater. In early stages this intermediate space is very extensive, penetrating between the brain and the otic capsule and also inside the trabeculae. In later stages it is reduced to a lateral space inside the descending process of the parietal, and in Stage V it is almost obliterated.

However, as de Beer pointed out, whether the space be extracranial or intramural, Gaupp's explanation of how the four eye-nerves come to have a common exit through the foramen orbitale magnum still holds good.

The cartilaginous process of the trabecular plate, my basitrabecular process, and Gaupp's bone associated with it have been the subject of much controversy in ophidian literature. Parker's (1878) description of *Tropidonotus* and Rathke's (1839) of *Coluber* both mention an alisphenoid cartilage in this region. Peyer (1912) says of this structure in the viper: 'Das Alisphenoid entsteht in der Gegend der Incisura prootica

des Primordialcraniums als Ersatzknochen mit Unterdrückung der knorpligen Präformation' (p. 607).

At one stage he found a short strip of cartilage jutting forward from the edge of the incisura antotica which he regarded as a possible vestige of a previous cartilaginous wall, but the bone he believed to be merely an extension of the pro-otic bone. The observed facts are evidently similar to those I have found in *Leptodeira*.

De Beer (1926) discusses the problem very fully. My observations on *Leptodeira* confirm his on *Pseudechis* and *Tropidonotus*. He illustrates the cartilaginous process from the trabecular plate, and *Tropidonotus* has an ossified column in close contact with the cartilaginous process. It separates the maxillary and mandibular branches of the trigeminal from the profundus, as in Stage IV of *Leptodeira*. He points out that the structure cannot be a pila antotica because it lies behind the profundus; it cannot be a pila lateralis as in *Amia*, for it is situated median to the vena capitis lateralis. He considers that it cannot be a processus ascendens, because it does not arise from and has no relations with the pterygoquadrate.

I do not consider this a serious objection. In the snake the quadrate is merely a rod of cartilage situated unusually far back. It has neither basal nor ascending process at any stage; evidently the anterior portion of the palatoquadrate cartilage has been lost or separated. The cartilaginous process has the relations of a basitrabecular process. It is a lateral projection of the trabecular plate immediately in front of the otic capsule. The palatine nerve emerges behind it and runs forward under its ventral surface. The vena capitis lateralis is dorsal to it. The bony column associated with it has the relations of an ascending process. It is lateral to the original cranial wall as shown by the prefacial commissure and basal plate and it is lateral to the vena capitis medialis. It must represent the outer wall of Gaupp's cavum epiptericum (1910). The space between the dura mater which is occupied by the vena capitis medialis and the trigeminal ganglion would be the cavum epiptericum. It is

situated in front of the maxillary and mandibular branches of the trigeminal which pass out behind it. It separates them from the profundus nerve which runs medially to it and passes out anterior to it. The bone in fully developed condition, as already described, has the relations of an epipterygoid, being external to the profundus and tending to grow back over the pro-otic and facial foramen. I cannot agree with Peyer that the bone is merely an extension of the pro-otic. It appears first in contact with the process of the trabecular plate, and there is a wide gap between it and the otic capsule which is overgrown in later development.

The epipterygoid bone, being an ossification of the ascending process of the palatoquadrate, is a replacing bone, and this bone in the snake is apparently never performed in cartilage. It is, however, conceivable that the cartilaginous stage has been suppressed and that the bone is laid down in the dense mesenchyme which represents the cartilaginous stage. This bone is of a different character from the descending process of the parietal. The latter is an additional outside wall; nerves do not pierce it, but pass round its anterior border. But this small bone in the region of the incisura antotica is pierced by the maxillary and the mandibular just as the replacing bone would be.

I see no serious objection to regarding this bone, Gaupp's bone, Parker's and Rathke's alisphenoid, Peyer's pro-otic extension, and de Beer's post-profundus laterosphenoid, as the homologue of the true reptilian epipterygoid.

I do not think it can be looked upon as in the nature of a laterosphenoid. As already pointed out, observations on *Leptodeira* show it to be lateral to the original line of the cranial wall, on the outer wall of the cavum epipticum.

In reference to this problem a series of sections of a very young night-adder embryo, still in the procartilaginous and thickened mesenchyme stage, were very interesting. They showed dense mesenchyme in a continuous strip lateral to the otic capsule, from the quadrate to the trabecular plate. It would suggest a single palatoquadrate structure.

Ethmoidal Region.

The ethmoidal region, as in snakes generally, is incomplete and delicate. The exceptionally light framework is evidently correlated with the excessive mobility of the jaws. It consists of a pair of cone-shaped cartilaginous capsules fused with the anterior extremity of the nasal septum. The nasal septum is a continuation of the fused trabecular rods, from the axis of which it inclines strongly downwards. It is a strong triangular rod of well-developed cartilage at its transition region, but rapidly narrows into a low vertical plate. Both dorsal and ventral edges are free throughout the greater part of its length. From this point forward the septum decreases rapidly in height and terminates against the premaxillary bone. It has no crista septi for the support of the septomaxillary bone, as Born (1888) reported for *Tropidonotus*.

The nasal capsules protrude in front of the septum, diverging from one another, and terminate in dome-shaped structures which form the end of the snout. Each capsule may roughly be divided into two regions; the posterior is rather broader than the anterior, from which it is sharply marked off by the anterior limit of the conchal infolding. The anterior half has an uninterrupted roof, but its medial wall is incomplete except for the very limited area of fusion with the nasal septum. The fenestrae superiores of *Lacerta* (Gaupp, 1900), *Eumeces* (Rice, 1920), *Sphenodon* (Schauinsland, 1900, and Howes and Swinnerton, 1901) are absent, nor does Peyer (1912) figure them for *Vipera*. In this the snakes are similar to the crocodiles and turtles. In Shiino's figures (1914) of *Crocodylus* there are no fenestrae superiores, but the fenestrae narinae extend dorsally into the anterior tectum nasi. I have been able to confirm this for an embryo *Crocodylus* (90 mm. head-length). Kunkel (1911) describes a tectum nasi uninterrupted by fenestrae superiores in *Emys*, and they are apparently absent in the *Dermochelys*, *Chelone*, and *Chelydra* investigated by Nick. In a *Chrysemys* embryo of 20 mm. head-length I have examined there are no fenestrae

superiores, but the fenestrae olfactoriae extend a very long way forward.

Posteriorly, the tectum nasi of *Leptodeira* is incomplete, a pair of very large fenestrae extending forward through half the length of the capsules. They are separated from one another medially by the nasal septum and the posterior wall of the capsule forms the posterior boundary. There are no sphenethmoidal cartilages so that each dorsal fenestra corresponds to the coalesced fenestra olfactoria and fissura orbito-nasalis of *Lacerta*. Shiino (1914) describes a similar coalescence in the crocodile and calls the foramen the fenestra cribrosa. The olfactory nerve and ethmoidal branches of the trigeminus nerve pass in conjunction through the fenestra cribrosa into the nasal capsule.

A noteworthy and interesting condition in the snake is the fusion of the posterior wall of the capsule with the nasal septum. In *Leptodeira* the posterior wall broadens slightly into a vertical plate (fig. 8, Pl. 13) which must correspond to the planum antorbitale of *Lacerta*. It is situated some distance laterally to the nasal septum, but from its medial ventral edge a narrow band of cartilage passes backwards and upwards to fuse with the dorsal margin of the nasal septum.

I have observed the same fusion in an embryo *Ptyās*. Peyer (1912) describes the side-wall of the nasal capsule of an embryo *Vipera aspis* (70 mm. head-length) as fused posteriorly with the nasal septum. From his figure this is evidently the same connexion. In an embryo of 125 mm. head-length he reports that the planum antorbitale is entirely lacking and in the figure of this later embryo the capsule is quite free from the septum nasi posteriorly. But in *Leptodeira* the range of embryos from about one month's age to two weeks before hatching all show complete fusion.

Other reptiles, with a single exception, are all described as showing complete freedom of the nasal capsules from the nasal septum posteriorly. Shiino (1914) describes the planum antorbitale as uniting solidly with the nasal septum in *Crocodilus*, but Gaupp in 1905 had reported it as free. In the single

Crocodylus I have at my disposal, the planum antorbitale is firmly wedged against the nasal septum, but the line of contact between them is distinct. Whether this contact is a process of detachment or of secondary attachment it is impossible to say from a single specimen. Apparently Shiino's twelve embryos of varying ages all showed complete fusion. He describes the nasal septum as thickened at the point of fusion and this might suggest a secondary connexion. In *Dermochelys* and *Chelonia*, Nick (1912) reports contact but no fusion between the planum antorbitale and the nasal septum, and in *Chrysemys* (Gaupp, 1905) and in *Emys* (Gaupp, 1905, and Kunkel, 1912) there is complete freedom. In the single *Chrysemys* I have been able to observe there is close contact without fusion.

With the exception of Shiino's crocodile the snake appears to be the only reptile in which the nasal capsule has a posterior commissure connecting it with the nasal septum. It might be a secondary attachment, an adaptation correlated with the delicate nature of the skeletal framework in the nasal region. Its persistence, however, throughout all stages of *Leptodeira*, with its early appearance and subsequent atrophy in the viper, suggest that it may be the primary condition.

Gaupp, however, regarded the posterior freedom of the nasal capsules from the nasal septum as the primary condition. In discussing the attachment of capsule in some mammals, he states his belief that the attached condition is a secondary modification, emphasizing the free condition of the reptilian ancestors as evidence of this (1910).

Kunkel observed that the connexion between the paraseptal cartilage and nasal septum in *Emys* is definitely a secondary modification, and concluded that in the primary condition the capsular wall is free from the nasal septum posterior to the *zona annularis* (1911).

The planum antorbitale of *Leptodeira hotamboia* has no maxillary processes.

The paries nasi are well developed and pass over uninterruptedly from the tectum nasi. Anteriorly there is a large

ventral gap, corresponding to the fenestra narina, but owing to the incompleteness of the capsular floor it is an open incisure, not a complete foramen. The processus alaris superior and processus alaris inferior are present as slight projections of the wall of the fenestra narina, and between them is situated the external nasal aperture.

The lateral wall in the posterior half of the capsules is complicated by the conchal infolding, which is in the form of an inverted trough open anteriorly, ventrally, and posteriorly. The extraconchal recess projects very slightly in front of the aditus conchae, so that the sulcus terminalis is very shallow. The lateral wall of the recess is complete, and there is no sign of the fenestra lateralis of Gaupp's *Lacerta* in any of my *Leptodeira* embryos.

The infolding of the paries nasi takes place early in development, and its invasion by the external gland is later. As pointed out by Rice (1920), this speaks strongly in favour of Born's theory (1879 and 1888) of the secondary relation of the gland to the concha, the folding of the olfactory epithelium being the active factor in the formation of the conchal infolding.

The nasal capsules are almost completely open basally, the entire floor being represented by the cartilaginous cup supporting Jacobson's organ. The cup is isolated from the rest of the cartilaginous skeleton. There is no anterior connexion with the cartilagine cupulares, as reported by Born (1888) for *Tropidonotus*. There is no connexion with the nasal septum. Laterally, fragments of histologically young cartilage and thickened tissue pass from the cup to the side-wall of the capsule, but there is no complete band as in *Lacerta*. This agrees with the condition found by Peyer (1912) in *Vipera*, but Born (1888) reports a continuous procartilaginous strip which later breaks down.

The isolated cup is supported by the prevomer and septomaxilla, which completely surround Jacobson's organ. The posterior portion of the cartilaginous cup protrudes up into the gland as a swollen knob, the concha. There are no paraseptal cartilages. The posterior edge of the cup is continued backwards

as a strip of cartilage which stretches to the choanae. A second cartilaginous strip runs parallel and lateral to the first process and unites with it posteriorly to form a plate of cartilage for the support of the nasal passage. The plates of the two sides approach one another very closely. Peyer describes similar cartilages in *Vipera* as the hypochoanal cartilages. Born (1888) describes them for *Tropidonotus* and *Vipera*, and identifies them with the hypochoanal cartilages of *Lacerta*. The sabre-shaped cartilages of *Python tigris* (Solger) are evidently of the same nature.

Owing to the incompleteness of the walls the foramina of the nasal capsule are not fully delimited. The fenestra narina and the fenestra cribrosa, the coalesced fenestra olfactoria, and fissura orbito-nasalis, have been described. Immediately behind its entrance into the fenestra cribrosa, the ethmoidal nerve (profundus branch of the trigeminal) divides into medial and lateral branches. The medial passes throughout the length of the capsule and emerges round the ventral anterior margin of the medial wall of the capsule (fig. 5, Pl. 13). No foramen apicale is delimited. The lateral ethmoidal emerges through a foramen epiphaniale and passes down the sulcus terminale (fig. 1, Pl. 12, and fig. 6, Pl. 13). The foramen epiphaniale is a slit-like aperture beginning slightly behind the aditus conchae. A very narrow strip of cartilage separates it from the fenestra cribrosa. If this broke down the course of the nerve would be similar to that described by Shiino for *Crocodylus* (1914). That is, the lateral ethmoidal would not enter the capsule at all but would pass over the roof of the capsule direct to the sulcus terminalis. In the same way the advehent aperture of the lateral ethmoidal, distinct from the fenestra cribrosa described by Nick (1912) for *Dermochylis* and *Chelonia*, is probably due to an extension of the cartilaginous wall of the roof to surround the nerve behind the foramen epiphaniale.

Mandibular Arch.

The dorsal division of the mandibular arch, the palatoquadrate cartilage, is represented in snakes by the long slender

quadrate bar. In Stage I of *Leptodeira* the quadrate is a narrow vertical plate of cartilage. Its articular facet for Meckel's cartilage is saddle-shaped. Correlated with the freedom of movement of the quadrate, it has no fusion with the wall of the skull. It is ligamentously attached to the so-called squamosal bone, the supratemporal of Thyng. The quadrate has no obvious basal or ascending processes. In a previous section on the orbito-temporal region of the skull, it was assumed as probable that this region of the palatoquadrate is represented by the epipterygoid bone, the cartilaginous stage of which is suppressed.

With advance in age of the embryo the distal end of the quadrate migrates backwards, until the slender elongated bar makes a very acute angle with the ventral division of the mandibular arch, Meckel's cartilage. This slender lower jaw extends forward in a gentle curve. The anterior ends of the two rami do not meet, a wide space intervening between them. The articulating surface with the quadrate is convex. There is a large retro-articular process.

Ossified Skull.

Replacing bones.—The stages at my disposal are not favourable to the study of the replacing bones. In Stage IV there is a little ossification in the occipital region. Well-developed exoccipital bones arch over the foramen magnum, but the supra-occipital region is unossified. The basi-occipital has an ossified occipital condyle. There is an interval of four or five weeks between Stages IV and V, and in the fifth stage ossification is so far advanced in the posterior region that the individuality of the bones is lost. The auditory capsule is completely ossified but the elements, pro-otic, epiotic, and opisthotic could not be distinguished, nor was any light thrown on the problem of whether the epiotic and opisthotic are independent elements. In the side-wall of the orbito-temporal region there can be no replacing bones, beyond the possible epipterygoid, since the cartilaginous wall has all been lost. Ossification has begun in the basisphenoid region. The posterior ends of the trabeculae,

enclosed in forceps-like parasphenoid structures, have atrophied. The anterior portion of the skull, the ethmoidal region, is in Stage V still persistent cartilage.

Membrane Bones.—The secondary skull of investing bones is very strongly developed, and my observations on the membrane bones of *Leptodeira hotamboia* confirm those of Peyer on *Vipera aspis*. The bones make their appearance at a very early stage, long before any sign of ossification in the cartilaginous cranium.

There are well-developed paired parietals, but they do not at first extend far enough toward the dorsal middle line to roof over the brain. The strongest development is a longitudinal strip over the summit of the auditory capsule. It extends a short distance down the mesial surface of the capsule between it and the brain. A short projection grows out over the anterior semi-circular canal. In front of the otic capsule the parietals have descending processes which form side-walls to the skull and compensate for the lack of cartilaginous wall. In Stage I the descending processes are not very extensive, but later they grow right down to the trabeculae. The anterior margin forms the posterior boundary of the foramen orbitale magnum.

A postfrontal bone is situated externally to the descending process of the parietal at its anterior end. The postfrontal inclines strongly outwards and downwards, forming a shelf over the orbit.

The frontals also are well developed, and have descending processes which form the side-walls of the anterior portion of the skull. These form the anterior margin of the foramen orbitale magnum. In the later stages of the *Leptodeira* embryos, the parietals and frontals are closely approximated in the roof of the skull, but there is a rounded gap in the middle line. They form an almost complete case for the brain in the orbito-temporal region. Each meets its fellow in the middle line. The descending processes of the frontals meet ventrally in the middle line, above the trabeculae. But between the descending processes of the parietals the parasphenoid forms the floor of the brain-case.

The prefrontal is a complicated bone. It forms a strongly convex arch over the side-wall of the cartilaginous nasal capsule. The broad base of the arch forms a strong support to the maxilla. The dorsal edge of the arch is in contact with the frontal forming a line of suture with it. In the viper Peyer describes an arch of the prefrontal extending dorsally between the nasal and frontal to meet its fellow of the other side, but in none of my *Leptodeira* specimens can I find such a development. Posteriorly the bone becomes outwardly concave extending under the eye.

The parasphenoid appears very much later than any other investing bone. It begins anteriorly as a vertical wedge of bone between the trabeculae. As the trabeculae separate posteriorly, the parasphenoid becomes a flattened plate of bone. Its lateral edges, forceps-shaped, enclose the trabeculae. It forms a floor over the hypophysial fenestra and extends back beneath the basisphenoid. The internal carotid artery runs forward in a channel between the parasphenoid and the basisphenoid, the parabasal canal, and enters the cranial cavity through notches in the antero-lateral corners of the crista sellaris.

The nasals penetrate deeply between the nasal capsules medianly, and in Stage V are quite large roofing bones to the capsules. The side-walls of the capsules anteriorly are unprotected by bone.

The septomaxilla and the prevomer surround Jacobson's organ. The prevomer forms the median and ventral walls of the bony capsule, and closes it posteriorly. The septomaxilla forms the lateral and dorsal walls and closes it anteriorly. Laterally the two bones overlap, the septomaxilla being outside the prevomer. A ridge of bone rises upwards from the median surface of the prevomer; it extends back along the nasal septum (fig. 9, Pl. 13).

In close contact with the septomaxilla and the prevomer anteriorly is the wedge-shaped, unpaired premaxilla. Its median ascending process extends up between the nasal capsules to meet the descending processes of the nasals.

The maxilla is a fairly long bone situated in a lateral position

beneath the skull. It is supported anteriorly against the prefrontal, and posteriorly it works against the transversum, or ectopterygoid. This latter is a flat bone, sloping gradually inwards to the pterygoid.

The pterygoid is an exceedingly long bone, extending from the jaw articulation forward in a medio-ventral position below the skull as far as the middle of the orbital region. In front it is loosely articulated against the palatine, a shorter bone extending beneath the nasal capsules. Posteriorly it works against the quadrate.

The maxilla, palatine, and pterygoid all bear teeth. They are all loosely articulated with one another and with the skull.

A long splint-like bone, generally known as the squamosal, develops upon the lateral aspect of the otic capsule. Anteriorly it is in close proximity to the parietal and extends back over the otic capsule between the prominences of the anterior and horizontal semicircular canals. Its ventral edge penetrates slightly between the quadrate and the wall of the otic capsule, and it does not extend over the surface of the quadrate at all. For this reason Thyng (1906) considers that it is a supratemporal and not a squamosal.

According to Thyng, the criteria for determining the homologue of the mammalian squamosal are its lateral position, overlying the otic capsule and the quadrate, and its contact with the quadratojugal. The bone in the Stegocephalia, which lies median to the squamosal and develops in close contact with the parietal, he calls the supratemporal. It is present with the squamosal in most of the primitive and extinct groups of Reptilia, but tends to be reduced in existing species. In Lacertilia it undergoes marked reduction, and in Sphenodon and Crocodilia is entirely lacking. The squamosal in all these is well developed.

According to Thyng's criteria, the bone called squamosal in the snake does more closely resemble a supratemporal. But in that case the squamosal must be entirely lacking, and this would be a unique instance in Reptilia. Snakes are peculiar

in having completely lost the temporal arcades and it is conceivable that the squamosal has disappeared with the quadratojugal, jugal, and postorbital.

Parker (1878) figured a small bone ventral to the squamosal (Thyng's supratemporal), which Thyng expected would be the true squamosal. But Gaupp failed to discover Parker's bone in his single *Tropidonotus* embryo. Peyer found it in none of his stages of the viper and I can find it in none of my stages of *Leptodeira*. But neither can I find any trace of the rest of the bones of the temporal arcades.

In the lower jaw of *Leptodeira hotamboia*, as in Gaupp's *Tropidonotus* and Peyer's *Vipera aspis*, the following investing bones may be distinguished: a dentary, a splenial, and a large composite bone. The dentary is situated dorsolaterally to Meckel's cartilage, a piece of which projects in front uncovered by bone. The splenial is a smaller strip along the medial side of Meckel's cartilage opposite the posterior portion of the dentary. The large composite bone is the posterior element extending from the dentary to the quadrate and enveloping Meckel's cartilage. In Stage I the articular element of the composite bone is unossified. A gonial and supra-angular are distinguishable components of the composite bone. A wide well-defined gap in the supra-angular allows for the passage of the mandibular branch of the trigeminal into the primordial canal. The chorda tympani passes round the posterior dorsal edge of the gonial into the primordial canal. Gaupp (1911) and Peyer (1912) both find a fourth separate element in the ophidian lower jaw. Gaupp calls it an angular, but Peyer does not find a separate element in the ventral position of an angular. He distinguishes a complementary, posterior, and dorsal to the splenial.

The skull as a whole has a very different appearance from the skull of the lizard (figs. 3 and 14, Pl. 12). The cranial region is a solid bony case. It affords a firm foundation against which the slender and loosely articulated palatal and jaw structures can work. This solid case is formed for the most part of the large parietal and frontal bones and their descending processes.

Inside this framework of membrane bones, the original cranial wall has undergone great reduction. As described in a previous section, the side-walls of the orbito-temporal region are entirely lacking. In the nasal region, too, a reduction of cartilage is correlated with a strong development of membrane bones, nasals, premaxilla, prevomers, septomaxillae, and prefrontals. A certain amount of movement is possible between the nasal region and the cranial case. Instead of the nasals being firmly wedged against the frontals, there is a gap between the bones which allows a certain amount of freedom.

The maxilla finds a firm anterior support against the massive prefrontal. Temporal arches are entirely lacking so that its posterior end is free except for a movable articulation with the pterygoid through the transverse bone. The palatine and the very much elongated pterygoid are freely movable. They are situated well away from the cranial floor. Posteriorly the pterygoid is supported against the quadrate with which it is freely movable. The pterygoid is not in contact with the basitrabecular process, which is relatively insignificant. In fact Gaupp says that only the large snakes, such as the python have basitrabecular processes (basipterygoid). The python has large processes against which the pterygoids are supported.

During development the quadrate shifts back along the squamosal (Thyng's supratemporal) and the posterior end of the latter is progressively raised until it forms a prominent ridge from the roof of the otic capsule. In snakes generally, the squamosal projects a long way behind the skull, but in Stage V the process has not extended beyond the otic region. The movability and length of the palatal and jaw-bones, the marked backward shifting of the quadrate and its loose articulation, and the movability of the nasal region all combine to produce a wideness of gape which enables the snake to seize and swallow comparatively large prey.

The unusual length of the temporal region, the excessive length of the basal plate between the facial foramen and crista sellaris already remarked, and the broad extent of the parietal side-wall, are probably correlated with the exceptional length

of the palatal and jaw structures, and may therefore be regarded as an adaptation to ensure a wider gape.

This account is in very close agreement with that given by Versluys (1912) for the python taken as a typical example of the snake. He classifies it as a mesokinetic type of skull, directly derivable from the condition in *Amphisbaenidae*, in which the movement is between frontals and parietals, not between frontals and nasals. Versluys regards the metakinetic condition of the lizard as primary, and believes that the mesokinetic character of the *Amphisbaenidae* and snakes is an adaptation from it for the particular habit of life and kind of food.

As described in a previous section the snake has no inter-orbital septum. The eyes are situated far apart outside the bony case of the parietals and frontals, and between the prefrontals and postfrontals; the eye-muscles and their nerves pass through the foramen orbitale magnum. There is no trabecula communis, but the paired trabeculae lie very close together for the anterior two-thirds of their length. This is an intermediate condition between the platybasic and the tropibasic types of skull. Gaupp (1908) and Versluys (1912) both consider that it is a secondary modification from the tropibasic reptilian condition. The eyes might be secondarily pushed apart and the trabecula communis separated into paired trabeculae with the formation of the broad cranial box.

The possibility of the secondary nature of the posterior attachment of the nasal capsules to the nasal septum has already been discussed. But the evidence in favour of the lacertilian origin of the *Ophidia* is debatable and insufficient to justify a dogmatic statement to that effect. It is conceivable that the highly specialized skull of the snake is fundamentally primitive and adapted from a more primitive ancestral type than the lizard. It is probable that from an investigation of the development of the less specialized snakes, such as the *Typhlopidae* and *Glauconidae*, their true nature might be read.

ABSTRACT.

The cartilaginous cranium of the snake, *Leptodeira hotamboia*, consists of basal plate and trabeculae, otic, and nasal capsules.

The crista sellaris of the basal plate is situated exceptionally far in front of the otic capsules, and the basicranial fenestra lies entirely in an anterior pro-otic third of the basal plate.

The trabeculae converge but do not fuse in front of the fenestra hypophyseos. They run forward parallel to each other, and fuse to form the nasal septum in the nasal region.

The otic capsules show a large vestibular division and a smaller cochlear portion. Posteriorly each capsule is separated from the basal plate by the fissura metotica. This fissure is divided into a small anterior medial opening of the recessus scalae tympani and a posterior jugular foramen. The vagus nerve passes through the posterior division, but the jugular vein, as in reptiles generally, passes out through the foramen magnum. The fenestra cochleae, an aperture of the cochlear capsule, faces towards the recessus scalae tympani.

The fenestra cochleae of the reptile is compared with the fenestra rotunda of the mammal, and it is found that the two are not homologous. The apertura lateralis of the recessus scalae tympani of the reptile is the homologue of the fenestra rotunda of the mammal. The secondary tympanic membranes in the two classes correspond morphologically as well as physiologically.

This conclusion does not confirm Gaupp's hypothesis (1900), but is in keeping with the earlier suggestion of Versluys (1898).

The columella auris consists of foot-plate and shaft. The distal end of the shaft is bent sharply backwards, is elongated, and is in contact with the quadrate. A small nodule at the distal end ossifies separately from the columella as a process of the quadrate. This is probably an intercalare, and the distal bent end of the columella would then represent the dorsal process of the lizard.

There is no interorbital septum. The cartilaginous side-walls

in the orbito-temporal region are lacking, and are compensated for by strong downgrowths of the parietals and frontals. The eye-muscles and their nerves are gathered together in the space behind these bones and pass out through a common opening, the foramen orbitale magnum.

A small basitrabecular process projects laterally from the trabecular plate. It supports a small bone situated in the side-wall of the skull over the trigeminal incisure and the facial foramen. The bone is not performed in cartilage, but its relations to the nerves and blood-vessels show it to be an epipterygoid. This basitrabecular process and epipterygoid evidently correspond to the so-called 'alisphenoid' of Parker (1878) and Peyer (1912).

The nasal capsules are delicate and incomplete. There are large conchal infoldings. The cartilaginous cup of Jacobson's organ is isolated from the rest of the nasal skeleton. A small planum antorbitale is present, and is attached to the dorsal edge of the nasal septum by a posterior commissure.

Membrane bones are strongly developed. Parietals, frontals, and parasphenoid form a strong bony case which gives a firm foundation for the working of the slender and loosely articulated palatal and jaw structures.

LETTERING OF FIGURES.

ab., abducens nerve; *ab.c.*, abducens canal; *a.l.*, apertura lateralis of recessus scalae tympani; *a.m.*, apertura medialis of recessus scalae tympani; *a.n.*, auditory nerve; *bc.f.*, basicranial fenestra; *b.p.*, basal plate; *b.p.g.*, basal plate gap; *bs.*, basisphenoid bone; *bt.p.*, basitrabecular process; *c.a.*, columella auris; *c.aq.*, cochlear aqueduct; *c.c.*, cochlear capsule; *c.d.p.*, columella distal process; *c.fp.*, columella foot-plate; *ch.t.*, chorda tympani; *co.*, concha; *c.s.*, crista sellaris; *d.p.*, parietal descending process; *d.p.f.*, frontal descending process; *d.m.*, dura mater; *ec.*, ectopterygoid; *eth.l.*, ethmoidalis lateralis; *eth.m.*, ethmoidalis medialis; *f.*, frontal; *f.c.*, fenestra cochleae; *f.cri.*, fenestra cribrosa; *f.ep.*, foramen epiphaniale; *f.hg.*, hypoglossus foramen; *f.hy.*, fenestra hypophyseos; *f.m.*, fissura metotica; *f.na.*, fenestra narina; *f.p.*, foramen perilymphaticum; *f.r.*, fenestra rotunda; *f.v.*, fenestra vestibuli; *G.b.*, Gaupp's bone; *gl.n.*, glossopharyngeus; *hy.f.*, hyomandibular nerve; *hy.c.*, hypochoanal cartilage; *i.a.m.*, membrane of incisura antotica; *i.c.a.*, internal carotid

artery; *J.o.c.*, Jacobson's organ capsule; *j.v.*, jugular vein; *M.c.*, Meckel's cartilage; *md.t.*, mandibular nerve; *m.t.*, maxillary nerve; *m.t.s.*, membrana tympani secundaria; *mx.*, maxilla; *n.*, nasal bone; *n.c.*, nasal capsule; *n.s.*, nasal septum; *o.a.*, orbital artery; *o.c.*, otic capsule; *ol.n.*, olfactory nerve; *o.n.*, oculo-motor nerve; *op.n.*, optic nerve; *pa.*, parietal bone; *pal.*, palatine bone; *p.a.i.*, processus alaris inferior; *p.a.s.*, processus alaris superior; *pas.*, parasphenoid; *pb.c.*, parabasal canal; *p.c.*, prefacial commissure; *p.f.*, palatine nerve; *pg.*, pterygoid bone; *pit.*, pituitary vein; *p.intr. p.mtr.*, processus intraperilymphaticus; *pl.ant.*, planum antorbitale; *p.n.c.*, posterior nasal commissure; *post.f.*, postfrontal bone; *pr.*, premaxilla; *pre.f.*, prefrontal bone; *p.s.*, perilymphatic sack; *p.t.*, profundus nerve; *pv.*, prevomer bone; *q.*, quadrate; *r.s.t.*, recessus scalae tympani; *sm.*, septomaxillary bone; *sq.*, squamosal bone; *st.*, stylohyale; *t.g.*, trigeminal ganglion; *t.n.*, trochlear nerve; *t.p.*, trabecular plate; *tr.*, trabecula; *ty.d.*, tympanic diverticulum; *v.*, vagus nerve; *v.c.l.*, vena capitis lateralis; *v.c.m.*, vena capitis medialis.

EXPLANATION OF PLATES 12 AND 13.

PLATE 12.

Fig. 1.—Reconstruction of the skull of *Leptodeira hotamboia*—dorsal aspect. Membrane bones have been removed to show relation of nerves and blood-vessels to the cartilaginous parts.

Fig. 2.—The same—lateral aspect.

Fig. 3.—Reconstruction of the skull—ventral aspect, with the membrane bones added as they appear in Stage V.

Fig. 4.—The same—lateral aspect.

PLATE 13.

Fig. 5.—Transverse section through nasal region of Stage I, 1-3-7, showing the exit of the medial ethmoidal nerve.

Fig. 6.—Transverse section through nasal region of Stage III, 3-1-1, showing the foramen epiphaniale.

Fig. 7.—Transverse section through nasal region, Stage IV, 2-3-4, showing vestiges of zona annularis.

Fig. 8.—Transverse section through nasal region, Stage IV, 3-3-2, showing the planum antorbitale and posterior commissure.

Fig. 9.—Transverse section through nasal region of Stage V, 3-1-7, showing the relations of the membrane bones to the cartilaginous parts.

Fig. 10.—Transverse section through the region of the incisura antotica at Stage IV, 9-1-1, showing the basitrabecular process and the rudiment of Gaupp's bone.

Fig. 11.—Transverse section slightly posterior to the last section, Stage IV, 9-2-6. It shows the margin of the trabecular plate and the membrane which bounds the *cavum epiptericum* laterally.

Fig. 12.—Transverse section through the orbitotemporal region, Stage III, 7-2-4. It shows the intermediate space between *dura mater* and the descending process of the parietal. The *profundus* nerve and the *vena capitis medialis* are situated in this space.

Fig. 13.—The same a few sections further back, Stage III, 7-2-10. The *abducens* nerve is seen in a canal on the upper surface of the basal plate.

Fig. 14.—Transverse section of Stage V, 13-2-5, through the region of the *incisura antotica*, showing the condition in the fully ossified skull.

Fig. 15.—The same, a few sections further back, Stage V, 14-1-1. *Gaupp's* bone is seen.

Fig. 16.—The same, a little further back, Stage V, 14-2-7.

Fig. 17.—Transverse section through the posterior nasal region of the fully ossified skull, Stage V, 5-2-2.

Fig. 18.—Transverse section through the orbital region of the fully ossified skull, Stage V, 9-2-7.

Fig. 19.—Horizontal section through the otic region of Stage I, 4-2-8. It shows the backwardly bent *columella auris*, the distal end in contact with the quadrate.

Fig. 20.—Transverse section through the posterior otic region of the fully ossified skull, Stage V, 16-3-9. It shows the *intercalare*, *Parker's stylohyale*, in contact with the quadrate.

Fig. 21.—Transverse section through the otic region, Stage IV, 10-3-5. It shows the *fenestra cochleae* and *recessus scalae tympani*.

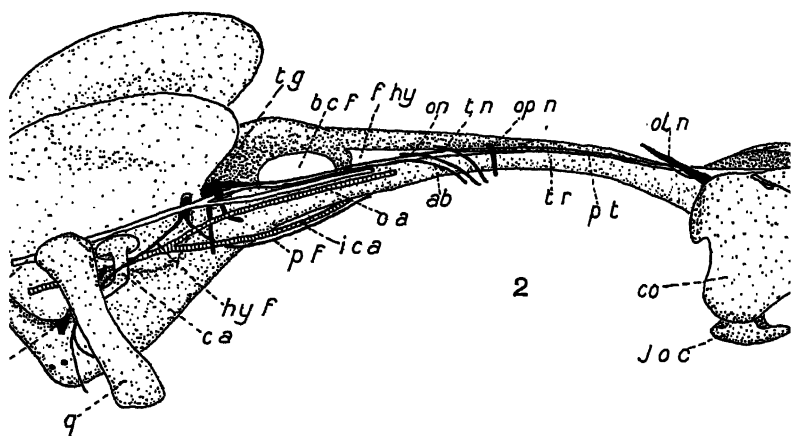
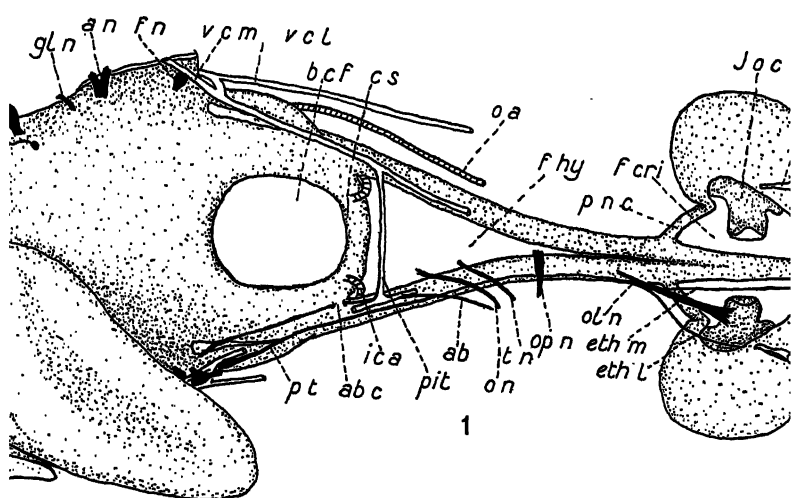
LIST OF LITERATURE CITED.

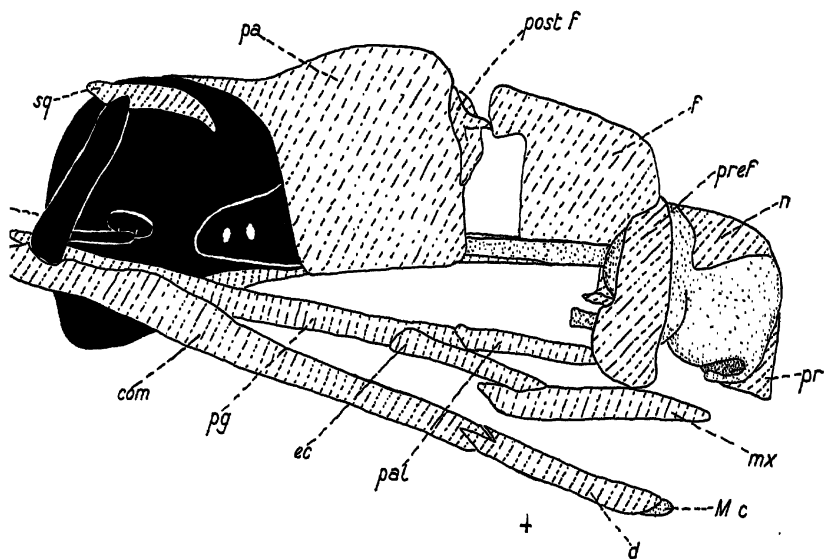
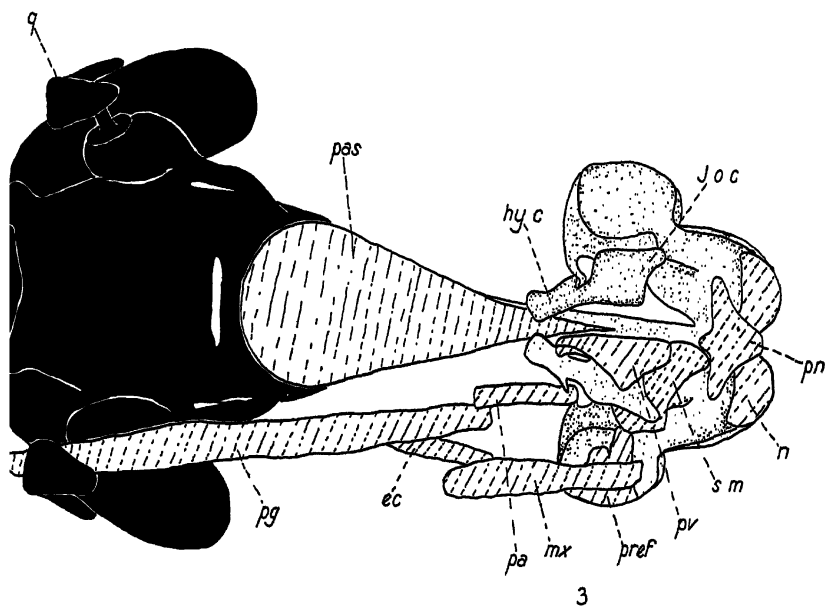
- de Beer, G. R. (1926).—"Studies on the vertebrate head. Pt. II. The orbitotemporal region of the skull", 'Quart. Journ. Micr. Sci.', vol. 70.
- Born, G. (1883).—"Die Nasenhölen und der Tränennasengang der amnioten Wirbeltiere III", 'Morph. Jahrb.', Bd. 8.
- Fischer, E. (1903).—"Zur Entwicklungsgeschichte des Affenschädels", 'Zeitschr. Morph. Anthropol.', Bd. 4.
- Fuchs, H. (1909).—"Ueber Knorpelbildung in Deckknochen, nebst Untersuchungen und Betrachtungen über Gehörknöchelchen, Kiefer und Kiefergelenk der Wirbeltiere", 'Arch. f. Anat. u. Physiol. Anat. Abt. Supplementband', 1909.
- Gaupp, E. (1900).—"Das Chondrocranium von *Lacerta agilis*", 'Anat. Hefte', Bd. 14.
- (1902).—"Ueber die *Ala temporalis* des Säugetierschädels und die *Regio orbitalis* einiger anderen Wirbeltierschädel", *ibid.*, Bd. 19.

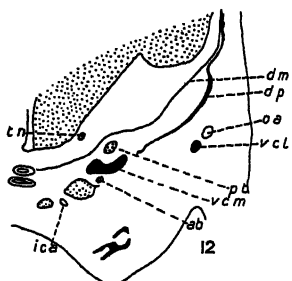
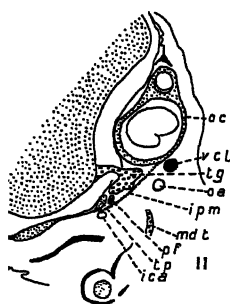
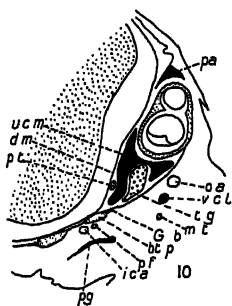
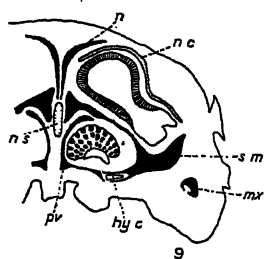
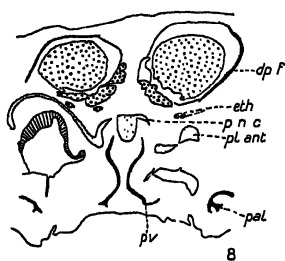
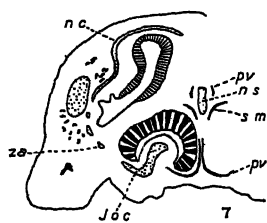
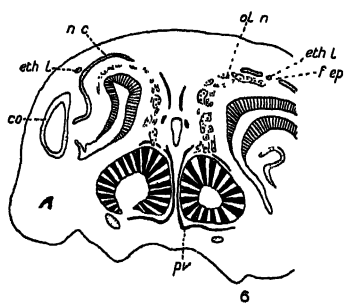
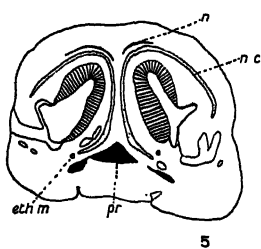
- Gaupp, E. (1906).—"Die Entwicklung des Kopfskeletts", Hertwig's 'Handbuch der Entwickl. d. Wirb.' Jena.
- (1908).—"Zur Entwicklungsgeschichte und vergleichenden Morphologie des Schädels von *Echidna aculeata*", 'Jenaische Denkschriften', Bd. 6, Teil 2.
- (1910).—"Die Verwandtschaftsbeziehungen der Säuger u.s.w.", 'Congr. Int. de Zool.', VIII, Graz.
- (1911).—"Beiträge zur Kenntnis des Unterkiefers der Wirbeltiere", 'Anat. Anz.', Bd. 39.
- Goldby, F. (1925).—"The development of the columella auris in the Crocodilia", 'Journ. Anat.', vol. 59.
- Goodrich, E. S. (1915).—"The chorda tympani and middle ear in reptiles, birds, and mammals", 'Quart. Journ. Micr. Sci.', vol. 61.
- Howes, G. B., and Swinnerton, H. H. (1901).—"On the development of the skeleton of the Tuatara, *Sphenodon punctatus*", 'Trans. Zool. Soc.', vol. 16.
- Kernan, J. D., jun. (1916).—"The chondrocranium of a 20 mm. human embryo", 'Journ. Morph.', vol. 27.
- Kunkel, B. W. (1911).—"Zur Entwicklungsgeschichte und vergleichenden Morphologie des Schildkrötenschädels", 'Anat. Anz.', Bd. 39.
- Macklin, C. C. (1914).—"The skull of a human foetus of 40 mm.", 'Amer. Journ. Anat.', vol. 16.
- Möller, W. (1905).—"Zur Kenntnis der Entwicklung des Gehörknöchelchens bei der Kreuzotter und der Ringelnatter u.s.w.", 'Arch. f. micr. Anat.', Bd. 65.
- Nick, L. (1912).—"Das Kopfskelett von *Dermochelys coriacea* L.", 'Zool. Jahrb., Abt. Anat.', Bd. 33.
- Okajima, K. (1915).—"Beiträge zur Entwicklungsgeschichte und Morphologie des Gehörknöchelchens bei den Schlangen", 'Anat. Hefte', 1. Abt., Bd. 53.
- Olmstead, M. P. (1911).—"Das Primordialcranium eines Hundeembryo", *ibid.*, Bd. 43.
- Parker, W. K. (1878).—"On the structure and development of the common snake", 'Phil. Trans. Roy. Soc.', 170, Pt. 2.
- Peyer, B. (1912).—"Die Entwicklung des Schädelskelettes von *Vipera aspis*", 'Morph. Jahrb.', Bd. 44.
- Rathke, H. (1839).—"Entwicklungsgeschichte der Natter". Königsberg.
- Rice, E. L. (1920).—"The chondrocranium of Eumeces", 'Journ. Morph.', vol. 34.
- Schauinsland, H. (1900).—"Weitere Beiträge zur Entwicklungsgeschichte der Hatteria", 'Arch. f. micr. Anat.', vol. 56.
- Shiino, K. (1914).—"Studien zur Kenntnis des Wirbeltierkopfes. I. Das chondrocranium von *Crocodylus* mit Berücksichtigung der Gehirnnerven und der Kopfgefäße", 'Anat. Hefte', Bd. 50.

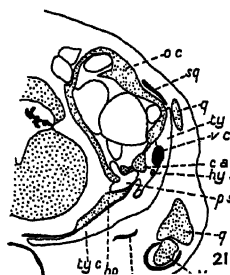
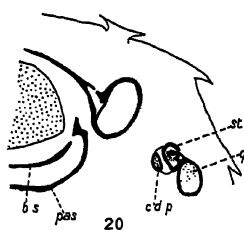
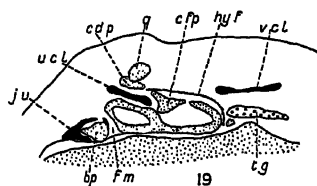
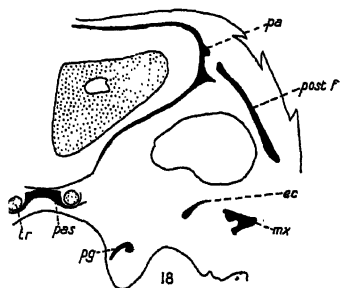
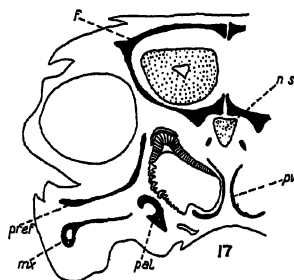
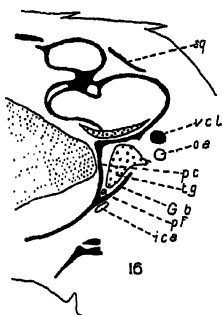
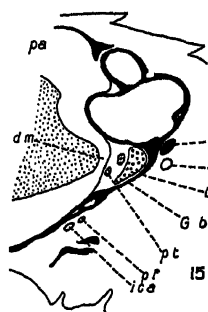
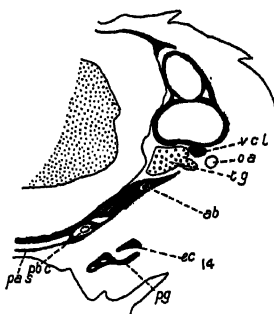
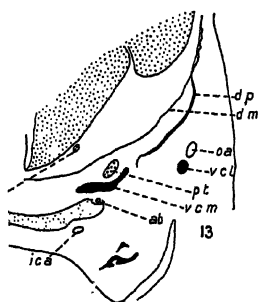
- Terry, R. (1917).—"The primordial cranium of the cat", 'Journ. Morph.', vol. 29.
- Thyng, F. W. (1906).—"Squamosal bone in Tetrapodous Vertebrata", 'Proc. Boston Soc. Nat. Hist.', vol. 32, no. 11.
- Versluys, J. (1903).—"Entwicklung der Columella auris bei den Lacertiliern", 'Zool. Jahrb., Abt. Anat.', Bd. 19.
- (1899).—"Die mittlere und äussere Ohrsphäre der Lacertilia und Rhyncocephalia", 'Zool. Jahrb.', Bd. 12.
- (1912).—"Das Streptostylie Problem", *ibid.*, Sup. XV, Spengel II.
- Voit, M. (1909).—"Das Primordialcranium des Kaninchens unter Berücksichtigung u.s.w.", 'Anat. Hefte', Bd. 38.
- Wyeth, F. J. (1924).—"On the development of the auditory apparatus in *Sphenodon punctatus*", 'Proc. Roy. Soc. London', Series B, vol. 91.













The Formation and Fate of the Operculum and Gill-chambers in the tadpole of *Rana temporaria*.

By

Gwendolen T. Brock, M.Sc., D.Phil. (Oxon.).

With 16 Text-figures.

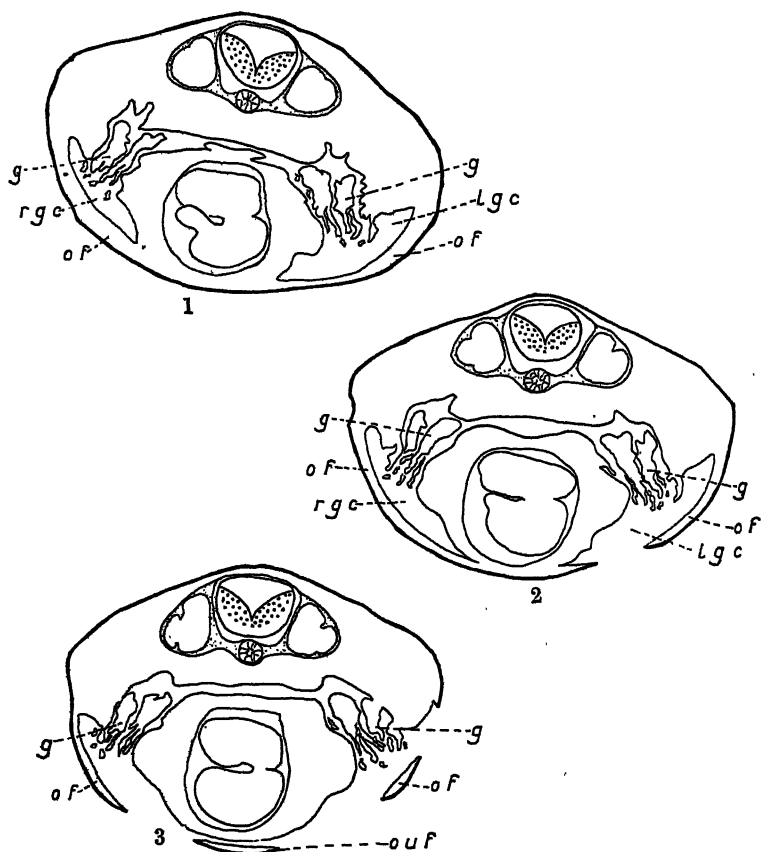
THIS work has been undertaken with the object of establishing the exact method of formation of the operculum and gill-chambers in the tadpole. It is also intended to follow up a suggestion of O. H. Latter (1923) that branchial respiration continues after metamorphosis by means of paired apertures immediately in front of the bases of the freed anterior limbs.

It is generally known that a fold of skin, the operculum, appears in front of the gill clefts shortly after hatching and grows back over the clefts, enclosing the gills in a gill-chamber. In *Rana* the right and left chambers communicate with one another ventrally, and the only opening to the exterior is a funnel-like opening on the left side of the tadpole. Text-book accounts, Balfour's 'Comparative Embryology', for instance, are very vague in defining how the communication between right and left chambers is effected, and in what way the right opening is obliterated. An exact account, in outline, is given by Milnes Marshall. He says that the operculum grows backwards and fuses with the body-wall along the ventral surface and on the right side, leaving an opening on the left side which communicates with both chambers. This account I have found to be quite correct.

I have examined a large number of serial sections of tadpoles, transverse and vertical, varying in age from the newly hatched tadpole to the metamorphosed frog, with four limbs, lungs, and external nares.

Text-figs. 1, 2, and 3 represent transverse sections through a young tadpole, soon after hatching. The operculum is forming.

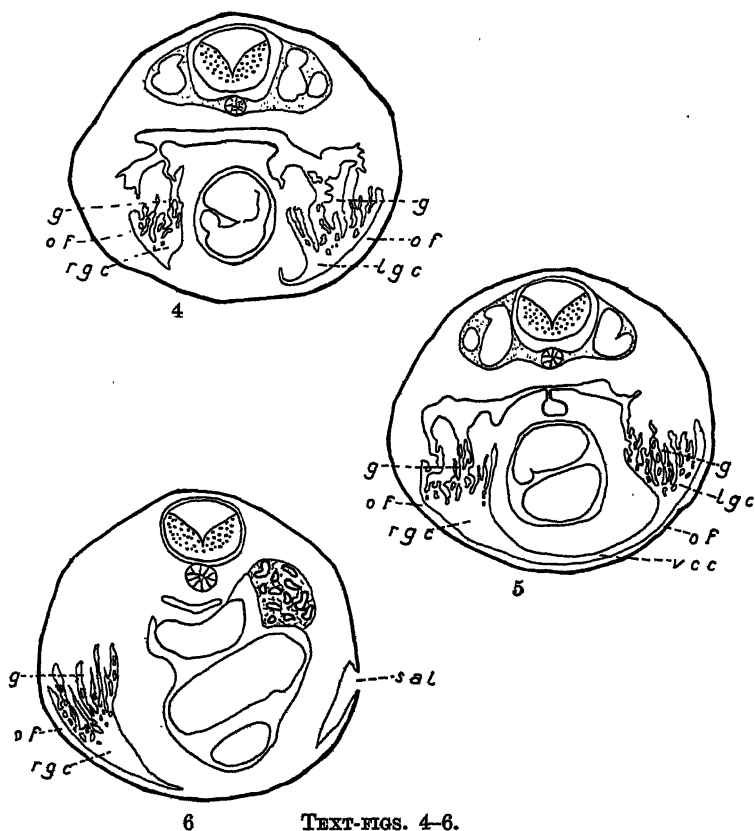
TEXT-FIGS. 1-3.



a.l., anterior limb; *b.c.*, branchial cavity; *co.*, coracoid; *ect.*, ectoderm; *e.g.*, external gills; *g.*, gills; *int.*, integument; *l.a.l.*, left anterior limb; *l.g.c.*, left gill-chamber; *o.*, operculum; *o.c.*, operculum complete; *o.f.*, opercular fold; *o.v.f.*, opercular ventral fold; *p.g.*, pectoral girdle; *r.a.l.*, right anterior limb; *r.g.c.*, right gill-chamber; *s.a.l.*, spiracular aperture, left; *s.b.a.*, secondary branchial aperture; *v.c.c.*, ventral communicating channel.

Text-fig. 1, the most anterior section, shows separate right and left cavities, closed off from the exterior by the lateral folds.

A few sections further back, Text-fig. 2, the fold has extended towards the mid-ventral line, while in Text-fig. 3, a ventral fold of skin lies below and separate from the body-wall. Tracing this ventral fold backwards in the sections, it is found to end freely.



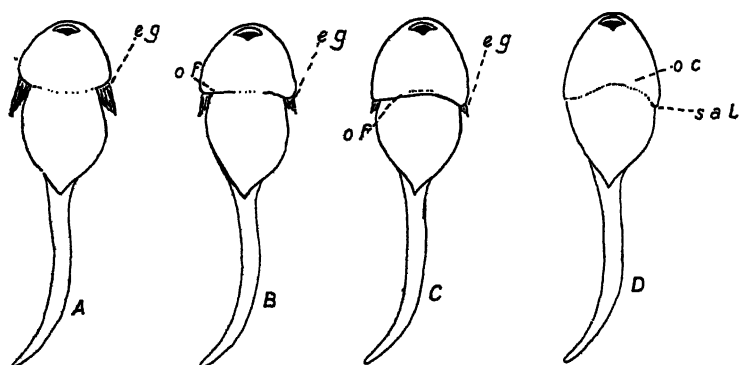
TEXT-FIGS. 4-6.

Both right and left cavities are wide open posteriorly. The operculum, then, must arise as a single fold of skin stretching in a convex sweep from the sides of the head right across the ventral surface of the body (Text-fig. 7 A). The lateral portions grow back much more rapidly than the ventral.

Text-figs. 4, 5, 6 are sections of a slightly older tadpole in
NO. 290

which the operculum is completely formed. The most anterior section, Text-fig. 4, again shows separate right and left cavities. In Text-fig. 5 these are joined by a ventral communicating space, and in Text-fig. 6 the ventral fold has fused again with the body-wall behind the communicating channel. Text-fig. 6 passes through the funnel-like left opening. The cavity on the right side, when traced back in the sections, is obliterated without any opening to the exterior.

A median vertical section, Text-fig. 11, shows the ventral



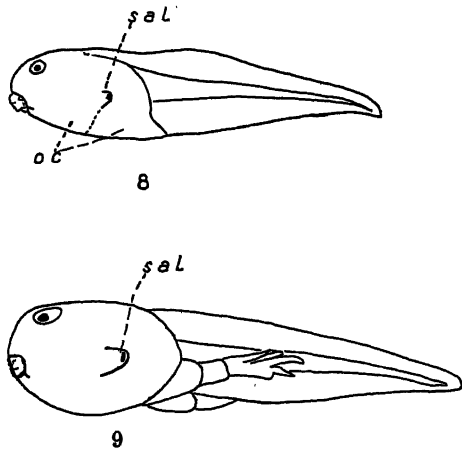
TEXT-FIG. 7.

communicating channel. The opercular fold of skin arises anteriorly, closes off the branchial channel, and fuses again with the body-wall.

Text-fig. 7 A, B, C, D of tadpoles seen from the ventral surface illustrate the formation of the operculum as determined from these sections. In A the gills are uncovered, but the incipient fold is suggested by the dotted line. In B the lateral portions of the folds have grown back rapidly and partially cover the gills. In C the lateral portions have extended still farther posteriorly; the left has outdistanced the right, and a tiny fold has arisen across the whole ventral surface. The complete opercular fold is free and unfused with the body-wall posteriorly. In D the operculum has fused with the body-wall, ventrally and on the right; a small opening remains on the left, drawn out into a funnel-like opening. It is shown in Text-fig. 8, a lateral

view of the same tadpole. Thus the branchial cavity consists of right and left chambers with a ventral communicating space, the whole cavity having been cut off from the exterior by the opercular fold of skin.

Since the branchial opening is at one time a single wide sweep from side to side across the ventral surface of the body, it is easy to understand that the definitive position may vary in different forms. *Bombinator* has a single ventral spiracle.



TEXT-FIGS. 8, 9.

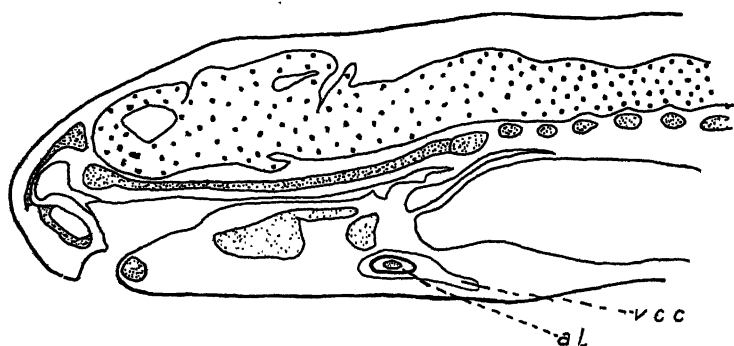
It would be expected that the fusion of the operculum with the body-wall would extend along the fold, converging from both extremities and leaving a ventral opening instead of one on the left side. In *Dactylethra* there are right and left openings according to Huxley (Balfour). It is possible that one might find the ventral communication as well, but it seems more probable that the extension of the opercular fold ventrally and the formation of the ventral communicating channel is correlated with the single branchial opening in *Rana*. One would, therefore, scarcely expect to find the ventral channel in *Dactylethra*.

The anterior limb-buds form on the walls of the gill-chambers. They protrude into the cavity, Text-fig. 10, the epidermis of the

limb being continuous with the lining of the gill-chamber. The lining of the whole branchial cavity is ectodermal in origin, but has become a single layered epithelium. As the limb-buds



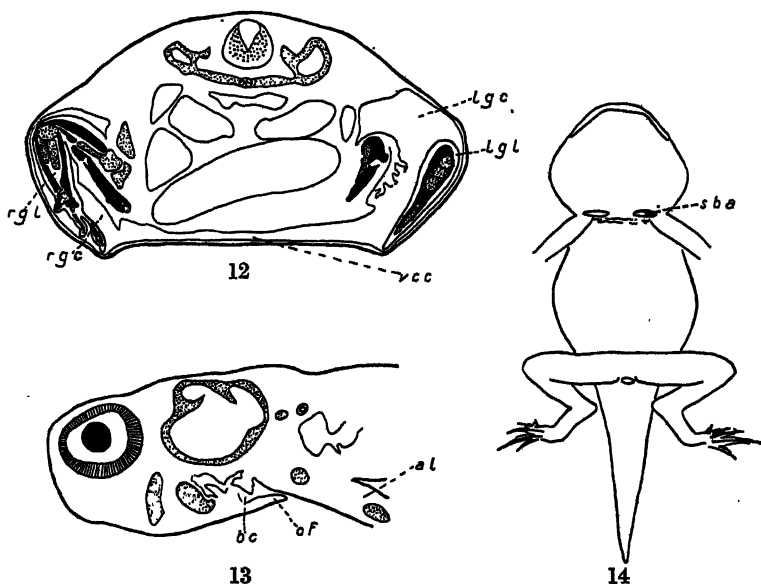
TEXT-FIG. 10.



TEXT-FIG. 11.

develop their epithelial covering assumes again the nature of a stratified epidermis. The buds grow into complete limbs within the gill-chambers. Text-fig. 12 is a transverse section of a tadpole shortly before metamorphosis. The tips of the digits and the elbows press against the wall of the operculum.

and it seems probable that both limbs at metamorphosis will break through the operculum. Milnes Marshall states that the right limb breaks through the operculum, but that the left emerges through the spiracle. O. M. Helff (1926) describes paired perforations due to histolysis of the operculum, but does



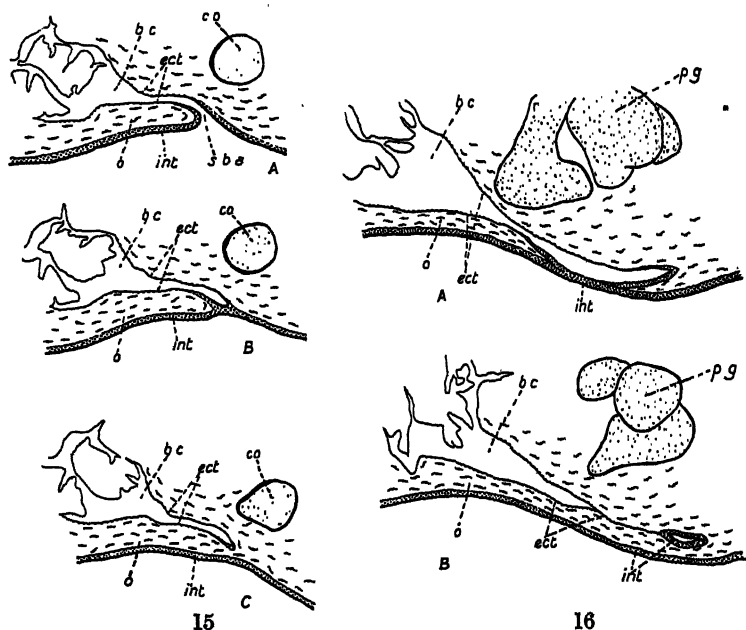
TEXT-FIGS. 12-14.

not say whether the spiracle shares in the freeing of the left anterior limb, or whether it closes independently.

O. H. Latter (1923) observed that tadpoles continue to breathe by means of gills, as well as by lungs, even after the forelimbs have appeared at metamorphosis, until the tail is completely absorbed. He observed paired crescentic openings with thickened lips, immediately in front of the bases of the freed anterior limbs, Text-fig. 13, and found that a branchial current passed in at the nostrils and out through these crescentic apertures.

My observations confirm those of Latter. Text-fig. 13 is a vertical section through a tadpole in which metamorphosis has taken place and the anterior limbs are freed. The section passes

through the left crescentic aperture, showing its thickened epidermal lip. The aperture commences immediately anteriorly to the base of the forelimb, but extends towards the mid-ventral line of the body. The apertures vary in size in different speci-



TEXT-FIGS. 15-16.

mens and on different sides of the same specimen. In no metamorphosed specimen is there a recognizable spiracle apart from the crescentic apertures.

At the lateral extremity of each crescentic aperture the operculum fuses with the skin of the limb, the outer surface of the opercular fold forming a continuous epidermal layer with the integument of the limb, Text-fig. 15 A, B, C. Helff (1926) described the continuity of the operculum with the integument of the limb after the atrophy of a shelf of integument which projects from the anterior surface of the limb as a result of the perforation process. Helff, however, regards the openings in

the opercular wall as purely perforations for the freeing of the anterior limbs and does not recognize them as functional respiratory apertures. At the medial extremity the crescentic apertures appear to be gradually closing. The anterior and posterior lips in Text-fig. 16 A, have come together and fused, and in Text-fig. 16 B, in a slightly more medial position, a small core of isolated epidermal cells may be observed below the surface of the body-wall. The outer surface of the operculum here forms the surface of the body-wall. The ectoderm of the lining of the branchial cavity is single layered, and the volume of the branchial cavity is being diminished by the growing together of the medial and lateral walls of the cavity. Median sections of this tadpole show that the ventral communicating channel, present before metamorphosis, has been entirely obliterated in this manner.

It would seem very likely then that the outer surface of the operculum persists as the permanent body-wall, and that the branchial cavity is obliterated by the sinking inwards of the operculum and the fusion of its inner surface with the inner wall of the branchial cavity, that is, with the original body-wall of the tadpole.

This work was carried out in the Department of Zoology and Comparative Anatomy, University Museum, Oxford.

LITERATURE CITED.

- Balfour, F. M. (1881).—‘Comparative Embryology’, vol. ii.
Helff, O. M. (1928).—“Studies on Amphibian Metamorphosis. I. Formation of the Opercular Leg Perforation”, ‘Journ. Exp. Biol.’, vol. 45.
Latter, O. H. (1923).—“An Overlooked Feature in the Four-legged Tadpole of *Rana temporaria*”, ‘Nature’ (February 1923).
Milnes Marshall, A. (1902).—‘The Frog. An Introduction’.

Yolk-formation in certain Tenthredinidae.

By

R. A. R. Gresson, B.Sc.

From the Department of Natural History, University College (University of St. Andrews), Dundee.

With Plates 14 and 15 and 3 Text-figures.

	PAGE
I. INTRODUCTION	345
II. PREVIOUS WORK	346
III. MATERIAL AND METHODS	349
IV. OBSERVATIONS	349
1. Golgi vacuoles and fatty yolk-formation	349
2. Golgi vacuoles in follicular epithelial cells	354
3. Golgi vacuoles in follicle cells	354
4. Mitochondria	354
5. Nucleolar extrusions and albuminous yolk-formation	355
V. DISCUSSION	358
VI. SUMMARY	360
VII. CONCLUSIONS	361
VIII. ACKNOWLEDGEMENTS	361
REFERENCES	361
EXPLANATION OF PLATES	363

I. INTRODUCTION.

THE present investigations were undertaken in order to determine the relationship of the oocyte and nurse-cell Golgi bodies and mitochondria to yolk-formation in the saw-flies, *Thrinax macula* Kl., *Thrinax mixta* Kl., and *Allantus (Emphytus) pallipes* Spin. (Enslin, 1). The oocyte nucleolar emissions of *Thrinax macula* and *Allantus pallipes*, and certain phenomena associated with the oocyte nucleolus of *Thrinax mixta*, have been already treated (Gresson, 4) and so has nucleolar budding in the latter

species (Peacock and Gresson, 14); the present contribution carries this last-mentioned investigation farther by describing in detail the staining reactions and phenomena connected with the nucleolar emissions of *Thrinax mixta*.

II. PREVIOUS WORK.

In a paper published in 1920 Gatenby and Woodger (3) dealt with the question of the relationship of Golgi apparatus and mitochondria to yolk-formation and showed that in the oogenesis of certain forms, e.g. *Patella*, &c., evidence exists to support the view that the Golgi apparatus takes part in deutoplasmogenesis, while in other forms, e.g. *Apanteles*, it has been claimed that the mitochondria are transformed into yolk. A large amount of work has been carried out since in the same direction, but as these contributions have been fully quoted by most other workers it is only necessary to refer briefly to some of the more recent findings.

In the eggs of most insects and related forms two kinds of yolk exist; a number of workers claim that one type, the albuminous, is derived from the cytoplasm or from nucleolar extrusions, while the other type, the fatty yolk, is formed by the transformation of the Golgi bodies.

On the other hand, certain investigators state that the albuminous yolk arises in relation to the Golgi bodies; thus Harvey, in a recent paper (5), makes this claim for *Carcinus*. He also points out the confusion which has arisen owing to the prevailing discordance of opinion.

Nath and Mehta (13), describing the egg of the fire-fly, *Luciola gorhami*, state that two kinds of yolk exist, albuminous and fatty. The former arises from nucleolar extrusions, the latter from the Golgi elements. In the undifferentiated germ-cells the Golgi apparatus exists in the form of about four rings lying on the edge of the nuclear membrane. The Golgi rings might be described as vacuoles 'with a sharp chromophilic rim and a central chromophobic substance (idiosome)'. On decolorization the osmicated fat spheres present a similar appearance and after further treatment appear

like clear vacuoles. These investigators conclude that free fat, not miscible with the general cytoplasm, is deposited in the interior of the Golgi rings, which in some cases may be saturated with fat before the egg is differentiated from the cells.

During the oogenesis of the spider, *Crossopriza lyoni* (Nath, 11), there are no nucleolar extrusions, the albuminous yolk arising independently in the cytoplasm. The Golgi elements in the youngest oocytes are in the form of 'vacuoles containing a watery and non-fatty fluid, and are embedded in the mitochondrial mass'. Later, the fatty yolk is formed by the 'deposition of fat not miscible with the general cytoplasm inside the Golgi vacuoles'.

Nath (8) believes that during oogenesis in the Chilopod, *Lithobius forficatus*, the nucleolar extrusions are associated with the formation of albuminous yolk. The juxta-nuclear Golgi apparatus of the young oocytes fragments and spreads through the cytoplasm, the granules being converted into fatty yolk. King (7), working on the same species, states that the nucleolar extrusions on reaching the cytoplasm increase by budding, 'the grains so formed eventually enlarging into yolk-spheres'. The Golgi bodies fragment and spread through the cytoplasm; 'the origin of the fatty yolk is doubtful, but it may be possibly connected with the Golgi apparatus'. This conflicting evidence has led Nath and Husain (12) to investigate the origin of yolk in the Scolopendrid, *Otostigmus feae*. In this type the nucleolar extrusions are few and disappear before the albuminous yolk arises independently in the cytoplasm. 'The Golgi elements exist in the form of vacuoles with watery and non-fatty contents', the fatty yolk being formed by a process of growth and deposition of free fat inside the Golgi vacuoles.

Rao (15) records an interesting condition in the ovum of the lemur, *Loris*. Nucleolar emissions arise from the plasmosome and initiate the formation of fat-globules, while the mitochondria and Golgi apparatus play a part in the origin of the yolk-spheres.

Nath (9 and 10), reviewing Parat's work on the Golgi vacuoles,

points out that, according to the latter, the Golgi elements in all vertebrate and invertebrate cells exist in the form of vacuoles, while the reticular appearance of the apparatus in the somatic cells of vertebrates is an artifact. The contents of these vacuoles are mostly liquid and their reaction acidic; hence their affinity to the basic neutral red.

Nath found that the eggs of *Crossopriza* (11) and of *Otostigmus* (12), stained with janus green or neutral red, contain vacuoles of two sizes, the smaller being Golgi vacuoles and the larger fatty yolk. The latter arise by growth and by the deposition of fat inside the former. The solid granular Golgi elements shown in fixed eggs he regards as artifacts due to the excessive precipitation of metallic silver or osmium inside the vacuoles. Gatenby (2) in a recent paper reviews the work of Hirschler, Monné, Voinov, and others; these investigators state that during spermatogenesis of certain forms, the vacuoles may be separated from the Golgi apparatus. Gatenby has shown that a similar condition exists in the male cells of *Cavia*, *Helix*, and *Abaxas*. Hence 'the vacuole is not the Golgi apparatus, but the associate or derivative of the Golgi cortex'.

From the above account it would appear that the behaviour of the vacuole system varies in male and female cells, in the male becoming separated from the Golgi apparatus, while in the female apparently the Golgi element and vacuole remain in association during oogenesis. Gatenby (op. cit.) believes 'that in such examples of oogenesis as that of *Daphnia*, the Golgi element is a cortex on the vacuole, and the division of the element brings about a division of the associated vacuole'.

It is worthy of note that Hibbard (6) describes Golgi vacuoles in the eggs of the Amphibian, *Discoglossus*, which stain with neutral red, later 'lose their capacity for being vitally stained', and give rise to yolk; 'the fat arises *de novo* in the cell independently of the mitochondria or vacuoles'.

To summarize: there is a large amount of evidence in favour of the views that the Golgi apparatus gives origin to fatty yolk, and that, in many cases, the origin of albuminous yolk appears to be attributable to nucleolar extrusions; it should be borne in

mind, however, that all workers are not agreed on the above points, as some claim a Golgi origin for albuminous yolk; only further work will clarify the position; as will be seen later, an important observation from the point of view of the present paper is that in *Crossopriza* and *Otostigmus* Golgi vacuoles are converted into fatty yolk globules.

III. MATERIAL AND METHODS.

The material for this paper was obtained from specimens of *Thrinax mixta* Kl., *Thrinax macula* Kl., and *Allantus* (*Emphytus*) *pallipes* Spin. (Enslin, 1), the two former species in February, March, and April 1929 from larvae, pupae and adults which had hibernated in the larval condition, the latter species from corresponding stages, in March and April 1929.

For an examination of the Golgi vacuoles, ovaries were dissected out in saline solution and subsequently stained with neutral red, mounted in a drop of stain or saline and examined. The above material was checked against ovaries fixed by the standard Mann-Kopsch, Kolatchev, and Da Fano methods. Fixation in 2 per cent. osmic acid was also found useful.

In the case of *Thrinax mixta* the phenomena associated with the nucleolar extrusions were worked out in ovaries fixed in corrosive acetic fixative and stained in Mann's methyl-blue eosin. In all cases the following procedure was adopted: the ovaries were dissected out in tap-water and immediately transferred to the fixative; sections were cut 3μ and 5μ in thickness.

IV. OBSERVATIONS.

1. Golgi vacuoles and fatty yolk-formation.

The phenomena associated with the Golgi vacuoles and yolk-formation were worked out in *Thrinax mixta* and *Thrinax macula* before the later stages of the *Allantus pallipes* material were examined. Consequently the following account refers to the former two species.

Mann-Kopsch preparations of the early undifferentiated cells at the proximal end of the ovarioles showed the Golgi element

as small round osmophil bodies situated chiefly round the nuclei (fig. 1, Pl. 14). On examining the young oocytes not fully separated from the adjoining nurse-cells, many of the bodies are seen to have increased in size (fig. 3, Pl. 14).

In neutral red preparations clear vacuoles are shown to occupy similar positions to those of the osmophil bodies in the undifferentiated cells and early oocytes (figs. 2 and 7, Pl. 14).

Furthermore, on decolorizing with turpentine, the dark bodies of the Mann-Kopsch material were clearly shown to consist of a vacuole with a chromophilic rim (fig. 11, Pl. 14) and, on prolonged treatment, the whole body appeared as a clear space; fixation for a short period in osmic likewise shows the chromophilic rim. Thus, it is evident that the osmicated bodies are in reality vacuoles and the Golgi element in all probability is represented by the chromophilic rim observed in decolorized material. In the early oocytes the Golgi vacuoles surround the nucleus and extend for some distance towards the periphery. With the growth of the oocyte the vacuoles increase in number, and in the older cells, just before yolk-formation, occur throughout most of the ooplasm. These stages are shown in figs. 4 and 6, Pl. 14. It should be noted that in figs. 7 and 8, Pl. 14, of neutral red preparations the cells and nuclei are larger and the Golgi vacuoles slightly smaller than in the fixed material.

The phases of yolk-formation were best revealed in Mann-Kopsch material. Thus, the onset of deutoplasmogenesis is marked by the appearance of small yolk-globules towards the periphery of the oocytes. These are yellowish or very faintly darkened in osmic preparations. They rapidly increase in size and at the same time certain of the Golgi vacuoles swell up to form large deeply osmophil spheres (fig. 22, Pl. 15). The latter are very resistant to the decolorizing action of turpentine, both types of vacuole are destroyed by acetic fixation, the position of the larger ones in the older eggs being marked by clear spaces situated among the yellow yolk-globules. The reaction of the large vacuoles to osmic and acetic fixation, and their non-preservation in oocytes treated by the Da Fano method, shows clearly that they are the fatty yolk-globules. The above facts,

together with the evidence of the existence of vacuoles intermediate in size between the small Golgi vacuoles and the large fatty yolk-vacuoles, leaves no doubt that the former are transformed into the latter at a certain stage of oogenesis. The change takes place, in all probability, by the deposition of free fat within the original vacuoles.

In eggs treated with neutral red the fatty yolk remains colourless or is faintly coloured according to the length of treatment in the vital stain. The ordinary or albuminous yolk-spheres first mentioned are easily distinguished owing to their yellow colour.

Shortly after the first appearance of the fatty yolk a noteworthy and peculiar condition is observable in the albuminous yolk-globules, many of the smaller spheres presenting a somewhat granular appearance. Most of these granules on closer examination proved in reality to be small vacuoles or droplets situated within the yolk-globules, the larger globules containing large 'vacuoles' varying in size and in number (fig. 14, Pl. 14; fig. 23, Pl. 15). In slightly older oocytes these appearances were more marked and the yellow yolk-globules seemed to have increased in number. In the fully formed eggs the albuminous yolk-spheres were very numerous and did not contain large 'vacuoles', although in some cases small 'vacuoles' were observed (fig. 24, Pl. 15).

This 'vacuolated' condition marks some stage in the development of the albuminous yolk of the mature egg. The appearance of the spheres suggests that multiplication may take place by the 'vacuolated' globules becoming broken up into smaller bodies, and although no such process was observed, certain facts would seem to give weight to this supposition. Thus there is a great increase in the amount of albuminous yolk immediately after the 'vacuoles' make their appearance, and furthermore, in the fully formed egg after yolk-formation has been completed, the larger 'vacuoles' were not shown, while the smaller ones were only present in a few globules.

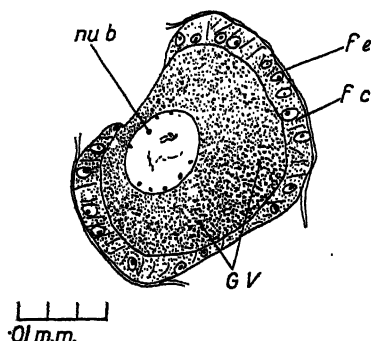
In the opinion of the writer there is no evidence to connect the Golgi vacuoles with the formation of albuminous yolk. The

latter first arises towards the periphery, regions of which are comparatively free from Golgi vacuoles. The origin of the albuminous yolk will be discussed in more detail later (pp. 357-8).

In the mature eggs the fatty yolk globules are not numerous, but those present are larger than in the earlier oocytes (fig. 24, Pl. 15). Some of the small Golgi vacuoles appear to remain unchanged and at this stage can usually be observed among the yolk-globules.

The behaviour of the Golgi vacuoles in the nurse-cells is

TEXT-FIG. 1.



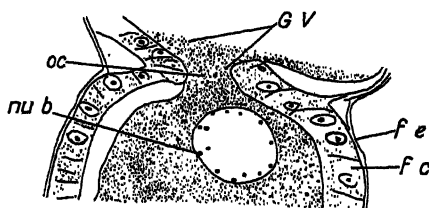
Young oocyte of *Allantus pallipes*, showing Golgi vacuoles, the most of which occur towards the posterior end of the oocyte. *f.c.*, follicle cell; *f.e.*, follicular epithelium; *G.V.*, Golgi vacuoles; *nu.b.*, nucleolar bud.

closely similar to that of those in the oocytes. In the early nurse-cells not yet separated into nutritive chambers the vacuoles have already increased in size and are conspicuous in both fixed and neutral red preparations; they are situated chiefly in the vicinity of the nucleus but may also occur in the cytoplasm towards the periphery (fig. 12, Pl. 14). With the growth of the cells they increase in size and number, and in the mature nurse-cells many are equal in size to the larger vacuoles observed in the oocytes before the onset of yolk-formation (fig. 18, Pl. 14). They are present in all the fully formed nutritive chambers and are, no doubt, carried into the egg by the cytoplasmic flow described in a former contribution (14). Although a large

amount of material was examined, in these two species the Golgi vacuoles were not seen passing through the connexion from the nutritive chamber to the oocyte, but this must take place as the nurse-cells are finally absorbed by the developing egg.

An examination of the *Allantus pallipes* material showed that the phenomena associated with the Golgi vacuoles and yolk-formation were similar to those of the other two species

TEXT-FIG. 2.



0.1 mm.

From *Allantus pallipes*, showing Golgi vacuoles situated in connexion between nutritive chamber and oocyte. *o.c.*, chamber-oocyte connexion; other lettering as in fig. 1.

of saw-fly described. Only one point of difference was noted: in the young oocytes the Golgi vacuoles lie more towards the periphery and become most numerous in the posterior part of the egg, thus leaving a comparatively clear space around the nucleus (Text-fig. 1). In two cases Golgi vacuoles appeared to be passing through the connexion from the nutritive chamber to the subjacent oocyte (Text-fig. 2).

To summarize: small Golgi vacuoles are present in the undifferentiated cells at the proximal end of the ovarioles; these increase in size in both oocyte and nurse-cell and ultimately give rise to the fatty yolk-globules of the mature egg; the Golgi vacuoles of the nurse-cells pass into the subjacent oocytes at a certain stage of oogenesis.

2. Golgi vacuoles in follicular epithelial cells.

The neutral red material revealed the existence of a number of brightly staining vacuoles situated in certain follicular epithelial cells (fig. 9, Pl. 14).

These cells were not evenly distributed but appeared to be present in groups throughout the follicular epithelium, being most numerous at the junction between nutritive chamber and oocyte. In the cells toward the posterior end of the ovarioles the number of these bodies within a cell was not so great, one or two large vacuoles being shown near the nucleus (fig. 10, Pl. 14). For these examinations osmic methods were not found as satisfactory as vital staining, but in a few cases the vacuoles after osmication were represented as dark bodies certainly similar in appearance to the Golgi vacuoles already described. They were also revealed in Da Fano material. These bodies are Golgi vacuoles but are not chemically identical with those of the nurse-cells and oocytes as evidenced by their affinity for neutral red.

3. Golgi vacuoles in follicle cells.

An examination of the follicle cells of the older oocytes in Mann-Kopsch preparations revealed the presence of small bodies similar to the Golgi vacuoles of oocyte and nurse-cells, and situated for the most part around the nuclei. These Golgi vacuoles were small and difficult to distinguish; they were shown best in Allantus pallipes material, where they appeared to be slightly larger and more numerous than in the other species (Text-fig. 3). Owing to the presence of vacuoles in the follicular epithelium and in the oocytes, these Golgi vacuoles could not be differentiated with certainty by means of vital staining.

4. Mitochondria.

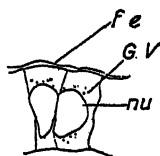
The mitochondria do not appear to play an important part in either the oocytes or nurse-cells. In stained Mann-Kopsch preparations they can be distinguished with difficulty as dots around the nuclei of the early cells (figs. 3 and 5, Pl. 14); in

the oocytes they become scattered through the ooplasm but cannot be followed in the late stages owing to the presence of yolk-globules. In the nurse-cells they appear to remain in the vicinity of the nucleus for a longer period, although many occur scattered in the cytoplasm (fig. 18, Pl. 14).

5. Nucleolar extrusions and albuminous yolk-formation.

The *Thrinax mixta* material fixed in corrosive acetic and subsequently stained in Mann's methyl-blue eosin revealed some

TEXT-FIG. 3.



┌───┐
0.1 m.m.

Follicle cells of *Allantus pallipes*, showing Golgi vacuoles round nucleus. *nu.*, nucleus; other lettering as in fig. 1.

remarkable facts in connexion with the nucleolar extrusions. While these phenomena bore a resemblance to the condition in the sister species, *Thrinax macula*, previously described by the writer (4), there are certain points of difference which are worthy of note. Thus the early oocytes contained a single basophil nucleolus of small size (fig. 15, Pl. 15), while in the oocytes slightly older, but not yet fully separated from the nurse-cells, an oxyphil nucleolus or plasmosome was present. The latter was not observed to arise by a process of differentiation from the basonucleolus as in *Thrinax macula* (4), but when present in the young cells was usually found in close proximity to the basophil nucleolus. In many instances this early stage was marked by the presence of vacuoles in the plasmosome and by buds outside it (fig. 16, Pl. 15), thus showing that budding commences shortly after the appearance of the

oxyphil nucleolus. Later, the basonucleolus, although still comparatively small, is seen to contain vacuoles (fig. 17, Pl. 15) which in all probability are the beginning of basophil extrusions. At this stage the plasmosome is active and in many cases a large oxyphil body is present which apparently has originated from it (fig. 21, Pl. 15).

In oocytes just before yolk-formation the oxyphil nucleolus is very active, oxyphil emissions being budded off and many of the latter occurring close to the nuclear membrane (fig. 18, Pl. 15). At this stage the basonucleolus is represented as a basophil body surrounded by slightly oxyphil material (fig. 21, Pl. 15). The basophil nucleolus was not observed liberating buds, but owing to its vacuolated appearance and to the presence of numerous basophil bodies surrounded by an oxyphil margin, there is no doubt that this process takes place. In a few cases large masses of oxyphil material were observed in the ooplasm and these contained a rounded basophil mass similar in appearance to the basophil nucleolar extrusions. One of these masses is shown in fig. 18, Pl. 15, together with a small body on the outside of the nuclear membrane; the latter is obviously an extrusion from the basonucleolus. The large body in the ooplasm is apparently a basophil extrusion undergoing some chemical change, causing the surrounding substance to become oxyphil. This supposition is borne out by the presence in the ooplasm of oxyphil bodies containing very faintly stained basophil material which appeared to be undergoing disintegration (fig. 19, Pl. 15). From the foregoing it would seem safe to conclude: (1) that the basophil extrusions, after passing through the nuclear membrane, undergo a change, the reaction causing the ooplasm in the immediate vicinity to become oxyphil; (2) that the basophil material finally becomes dissolved; this latter process being followed by the disappearance of the oxyphil substance.

The basonucleolus and basophil buds were observed in the older oocytes after the onset of yolk-formation (fig. 20, Pl. 15), but in no case were the extrusions seen outside the nuclear membrane. The oxyphil nucleolus when shown at this stage

appeared to be breaking up or giving rise to numerous buds, some of which occurred close to the nuclear membrane (fig. 20, Pl. 15).

Although this material did not reveal the presence of oxyphil emissions in the ooplasm all the observed facts point to such extrusion. Furthermore, a stained Mann-Kopsch preparation contained a small body in the ooplasm exactly similar in appearance to what was, in all probability, an oxyphil bud situated in the nucleus (fig. 6, Pl. 14), and in two other cases the same type of body was observed in stained Mann-Kopsch preparations of *Allantus pallipes* (fig. 25, Pl. 15). It should be borne in mind, however, that the appearance of the nucleoli presented in Mann-Kopsch material should not be greatly stressed, as, obviously, confusion might arise between basophil and oxyphil emissions. The bodies observed in the present case showed no resemblance to basophil extrusions and consequently would appear to lend weight to the opinion previously expressed by the writer (4) that the oxyphil buds of *Thrinax macula* and *Allantus pallipes* pass through the nuclear membrane, the difficulty of their detection in the ooplasm being due to their speedy dissolution.

The formation of the albuminous yolk commences shortly after the appearance of the basophil extrusions in the ooplasm. There is no evidence that either basophil or oxyphil emissions are directly transformed into yolk, but it would seem probable that the albuminous yolk-globules are formed as the result of an interaction between one or both types of extrusion and the cytoplasm.

To summarize: a basophil nucleolus is present in the early oocyte, and, later, a plasmosome appears; both basophil and oxyphil nucleoli give rise to emissions; the basophil type were observed in the ooplasm and there they undergo disintegration; the oxyphil emissions were not observed outside the nuclear membrane, but the evidence favours the view that they are extruded to the ooplasm; the basophil extrusions therefore, in all probability, play a part in albuminous yolk-formation, and the same may prove true for the oxyphil emissions; the

'vacuolated' appearance of many of the albuminous yolk-globules appears to be correlated with their increase in number.

V. DISCUSSION.

The phenomena described above show clearly that the dark osmophil Golgi bodies of the Mann-Kopsch preparations are in reality vacuoles with a chromophilic rim. These vacuoles appear to be similar to those described by Nath for the spider, *Crosso-priza lyoni* (11) and the Chilopod, *Otostigmus feae* (12). The present writer, however, is inclined to regard them as vacuoles with the original Golgi element forming the cortex. It has been shown conclusively by Gatenby (2) and others that Golgi body and vacuole become separated during spermatogenesis; hence the vacuole is a derivative of the Golgi body and not identical with it, as claimed by Parat. In oogenesis it would appear then that the Golgi element and vacuole remain in association.

In the species of saw-flies described here, it is evident that the Golgi vacuoles increase in size and finally give rise to the fatty yolk-globules. The process, in all probability, is one of growth and deposition of free fat inside the vacuoles, as claimed by Nath for *Crosso-priza* and *Otostigmus*. At a certain stage the number of Golgi vacuoles in the oocyte is increased by the passage into it of those from the nurse-cells.

Certain appearances would seem to suggest that fusion of Golgi vacuoles takes place, but this, however, was not observed in fresh material stained with neutral red. The suggestion is advanced only as a possible explanation of how larger vacuoles may be formed, though growth undoubtedly occurs at the same time. Again, the fatty yolk-globules in the fully formed eggs are not so numerous as the Golgi vacuoles in the younger oocytes, and, although certain vacuoles remain unchanged, these do not appear to be sufficient to account for their greater number in the earlier stages.

The nucleolar phenomena of *Thrinax mixta* resemble those of the sister species, *Thrinax macula*, previously described (4), but there are certain points of difference. In the

former species the nucleolus of the early oocytes is basophil, with an oxyphil nucleolus appearing later, which, however, was not observed to arise by differentiation from the original basophil nucleolus as in *Thrinax macula*. The presence in the ooplasm of several basophil bodies surrounded by oxyphil material is sufficient evidence to show that these bodies pass through the nuclear membrane. The basophil substance becomes less marked, dissolving finally and leaving the entire body oxyphil; the latter, however, disappears before ~~yolk~~-formation commences. This process is clearly similar to that described by Nath for *Lithobius* (8), where the nucleolar extrusions become amphophil and finally acidophil.

These large masses of nucleolar origin appear to be peculiar to *Thrinax mixta* as they were not observed in the other species of saw-flies in which nucleolar phenomena have been described (Gresson, 4).

The absence of oxyphil extrusions is probably due to their immediate dissolution in the ooplasm. Reliance cannot be placed on the apparent oxyphil extrusions shown in Mann-Kopsch preparations of *Thrinax mixta* and *Allantus pallipes*, as these may have been basophil buds which had undergone some change.

The facts revealed in the present investigation do not indicate that either type of nucleolar bud gives rise to fat globules, as claimed by Rao (15), for the oxyphil emissions of the lemur *Loris*.

The nucleolar origin of albuminous yolk has been claimed by several workers, but it should be borne in mind that certain investigators believe that the Golgi bodies have some responsibility for its origin. In the case of the saw-flies described in the present paper there is no evidence to indicate that the Golgi vacuoles play any part in albuminous yolk-formation; on the other hand, it appears highly probable that one or both types of nucleolar extrusion are concerned with this process.

Particular attention ought to be directed to the fact that the Golgi vacuoles, described by Hibbard (6) as giving rise to albuminous yolk, stained with neutral red, whereas those

observed by Nath (11 and 12) and the present writer did not stain but remained as clear vacuoles. This indicates a chemical difference between the two types. It is also of interest that two kinds of Golgi vacuoles, from the criterion of their reaction to neutral red, occur in the saw-fly ovariole; one, the unstainable vacuole of oocyte and nurse-cell, and the other, stainable, of the follicular epithelial cells.

There seems little doubt that the remarkable 'vacuolated' appearance which the albuminous yolk-globules assume shortly after the commencement of yolk-formation is intimately correlated with the growth and multiplication of these globules, and evidence of such is presented by their rapid increase and by the disappearance of the 'vacuoles' or droplets in the fully formed eggs.

VI. SUMMARY.

1. The Golgi vacuoles observed during the oogenesis of certain saw-flies were revealed by vital staining, osmic, and silver methods, in the undifferentiated cells at the proximal end of the ovarioles. After the differentiation of the nurse-cells and oocytes they increase in number and size. Later, those of the nurse-cells pass into the subjacent oocyte.

2. The Golgi vacuoles of the oocyte are converted into fatty yolk by the deposition of fat within the original vacuole.

3. Golgi vacuoles were revealed in the follicular epithelium and follicle cells; their function was not determined.

4. The mitochondria do not appear to play an important part in oogenesis.

5. In *Thrinax mixta* both oxyphil and basophil nucleolar buds occurred, the latter being observed as extrusions in the ooplasm. It is probable that the oxyphil buds also pass through the nuclear membrane. The nuclear phenomena of this species more closely resemble those of *Thrinax macula* than those of *Allantus pallipes*.

6. The phenomena associated with the oocyte nucleolar extrusions of *Thrinax mixta*, together with the facts observed previously in *Thrinax macula* and *Allantus*

pallipes, indicate that at least one type of nucleolar extrusion (basophil) takes part in the formation of albuminous yolk; both, however, may ultimately be shown to play this part. The 'vacuolated' appearance of many of the albuminous yolk-spheres is apparently connected, in some way, with their increase in number.

VII. CONCLUSIONS.

1. Fatty yolk in saw-fly oogenesis is formed by the conversion of the Golgi vacuoles. The properties and role of the Golgi vacuoles agree with those described for the invertebrates, *Crossopriza* and *Otostigmus*; they differ in staining properties from those of the vertebrate, *Discoglossus*, where they are stated to be converted into albuminous yolk.

2. Albuminous yolk is formed from basophil nucleolar extrusions, and possibly also from the oxyphil extrusions.

3. The characteristic 'vacuolated' appearance of many of the albuminous yolk-globules does not appear to have been previously recorded in invertebrate oogenesis.

4. It seems probable that the original Golgi element is represented by the cortex of the vacuoles of oocyte and nurse-cell.

5. The Golgi vacuoles of the follicular epithelial cells differ in staining properties from those of oocyte, follicle cell, and nurse-cell.

VIII. ACKNOWLEDGEMENTS.

I wish to express my thanks to Professor A. D. Peacock, in whose department these investigations were carried out, for research facilities, and for supplying me with saw-fly material.

REFERENCES.

1. Enslein.—"Die Tenthredinoidea Mitteleuropas", 'Deutsche Entom. Zeitschr.', Heft 7, 1912.
2. Gatenby, J. B.—"Study of Golgi apparatus and vacuolar system of *Cavia*, *Helix*, and *Abraxas* by Intra-Vital Methods", 'Proc. Roy. Soc.', vol. 104 'B', 1929.
- Gatenby, J. B., and Woodger, J. H.—"On the Relationship between Formation of Yolk and the Mitochondria and Golgi apparatus during Oogenesis", 'Journ. Roy. Micr. Soc.', 1920.

4. Gresson, R. A. R.—“Nucleolar phenomena during Oogenesis in certain Tenthredinidae”, ‘Quart. Journ. Micr. Sci.’, vol. 73, 1929.
5. Harvey, L. A.—“The Oogenesis of *Carcinus moenas* Penn., with special reference to Yolk-formation”, ‘Trans. Roy. Soc. Edin.’, vol. 56, Part I, 1929.
6. Hibbard, H.—“Cytoplasmic Constituents in the Developing Egg of *Discoglossus pictus* Otth.”, ‘Journ. Morph.’, vol. 45, 1928.
7. King, S. D.—“Oogenesis in *Lithobius forficatus*”, ‘Scient. Proc. Roy. Dubl. Soc.’, vol. 18, 1924.
8. Nath, V.—“Oogenesis of *Lithobius forficatus*”, ‘Proc. Camb. Phil. Soc. Biol. Sci.’, vol. 1, 1924.
9. ——— “On the present position of Mitochondria and the Golgi apparatus”, *ibid.*, vol. 2, 1926.
10. ——— “The Golgi Origin of Fatty Yolk in the light of Parat’s work”, ‘Nature’, vol. 118, pp. 767–8, 1926.
11. ——— “Studies in the Origin of Yolk. I. Oogenesis of the Spider, *Crossopriza lyoni* (Blackwell)”, ‘Quart. Journ. Micr. Sci.’, vol. 72, 1928.
12. Nath, V., and Husian, T.—“Studies in the Origin of Yolk. II. Oogenesis of the Scolopendra, *Otostigmus feae* (Pocock)”, *ibid.*
13. Nath, V., and Mehta, D. R.—“Origin of Yolk in the Eggs of *Luciola gorhami*”, ‘Nature’, vol. 119, pp. 13, 14, 1927.
14. Peacock, A. D., and Gresson, R. A. R.—“The Roles of the Nurse-cells, Oocytes, and Follicle-cells in Tenthredinid Oogenesis”, ‘Quart. Journ. Micr. Sci.’, vol. 71, 1928.
15. Narayan Rao, C. R.—“On the Structure of the Ovary and the Ovarian Ovum of *Loris lydekkerianus*, Fabr.”, *ibid.*

ADDENDUM.

Since this paper went to press Nath and Mehta have published a fuller account of the oogenesis of *Luciola gorhami* (‘Quart. Journ. Micr. Sci.’, vol. 73, 1929). Owing to the rapidity with which the Golgi vacuoles of the female primordial germ-cells blacken in 2 per cent. osmic acid, these workers conclude that the vacuoles contain free fat at an earlier stage than previously described for *Otostigmus* and *Crossopriza*.

In the case of the saw-flies dealt with in the present paper the staining reaction to neutral red would indicate that the vacuoles of the early oocytes and nurse-cells contain some free fat. These vacuoles, which give origin to the fatty yolk, remain unstained, while those described by Hibbard for *Discoglossus* (pp. 359–

60) and for certain Teleosts ('Journ. Anat.', vol. 61, 1927) as giving rise to ordinary yolk, are coloured by neutral red. The faint stain which some of the fatty yolk vacuoles appeared to possess after long immersion in neutral red (see p. 351) is probably due to the deeply stained surrounding ooplasm.

EXPLANATION OF PLATES 14 AND 15.

The drawings were made by means of a Zeiss camera lucida and a Watson 'Service' microscope. For all figures a Reichert $\frac{1}{10}$ objective was used. The eye-piece was a Hawksley No. 4 \times 10.

LETTERING.

AY, albuminous yolk; *b.n.*, basophil nucleolus; *b.n.b.*, basophil nucleolar bud; *f.e.*, follicular epithelium; *f.c.*, follicle cell; *G.V.*, Golgi vacuole; *M.*, Mitochondria; *n.*, nucleus; *nu.*, nucleolus; *nu.b.*, nucleolar bud; *nu.e.*, nucleolar extrusion; *o.b.*, oxyphil body; *o.n.*, oxyphil nucleolus; *o.n.b.*, oxyphil nucleolar bud; *oo.*, ooplasm; *v.ay.*, vacuolated albuminous yolk.

PLATE 14.

Figs. 1, 3-6, and 11-13 from *Thrinax mixta*. Figs. 2, 7-10, and 14 from *Thrinax macula*.

Fig. 1.—Undifferentiated cells at anterior end of ovariole, showing small Golgi vacuoles. Mann-Kopsch.

Fig. 2.—Undifferentiated cells. Neutral red.

Fig. 3.—Early oocyte at anterior end of ovariole, showing Golgi vacuoles and mitochondria. Mann-Kopsch.

Fig. 4.—Slightly older oocyte. Mann-Kopsch.

Fig. 5.—Later oocyte not fully separated from nurse-cells, showing increase in size and number of Golgi vacuoles. Mann-Kopsch.

Fig. 6.—Part of more fully formed oocyte. The Golgi vacuoles have increased in number and spread through the ooplasm. Nucleolar extrusion is shown in the ooplasm. Mann-Kopsch.

Fig. 7.—Early oocyte not fully separated from nurse-cells. The nucleus and cell are larger than in the corresponding stage in the fixed material. Neutral red.

Fig. 8.—Golgi vacuoles round nurse-cell nucleus. Neutral red.

Fig. 9.—Follicular epithelial cell showing Golgi vacuoles. Neutral red.

Fig. 10.—Follicular epithelial cell situated towards posterior end of ovariole, showing two large Golgi vacuoles. Neutral red.

Fig. 11.—Showing Golgi vacuoles in decolorized Mann-Kopsch material.

Fig. 12.—Nurse-cells at anterior end of ovariole. Mann-Kopsch.

Fig. 13.—Nurse-cell from fully formed nutritive chamber. Mann-Kopsch.

Fig. 14.—Late oocyte, showing Golgi vacuoles, fatty yolk and albuminous yolk. Neutral red.

PLATE 15.

Figs. 15–22 and 24 from *Thrinax mixta*. Fig. 23 from *Thrinax macula*. Fig. 25 from *Allantus pallipes*.

Fig. 15.—Early oocyte, showing basophil nucleolus.

Fig. 16.—Later stage, oxyphil, and basophil nucleolus present; the former giving rise to buds.

Fig. 17.—Showing oxyphil body which has originated from the oxyphil nucleolus.

Fig. 18.—Oocyte before yolk-formation. Oxyphil and basophil nucleolar buds shown; basophil extrusion outside nuclear membrane; large basophil body surrounded by oxyphil material shown in ooplasm.

Fig. 19.—Oocyte before yolk-formation. Oxyphil body with faintly basophil granules shown in ooplasm.

Fig. 20.—Oocyte after yolk-formation. Basophil nucleolus and bud; oxyphil nucleolar bud present.

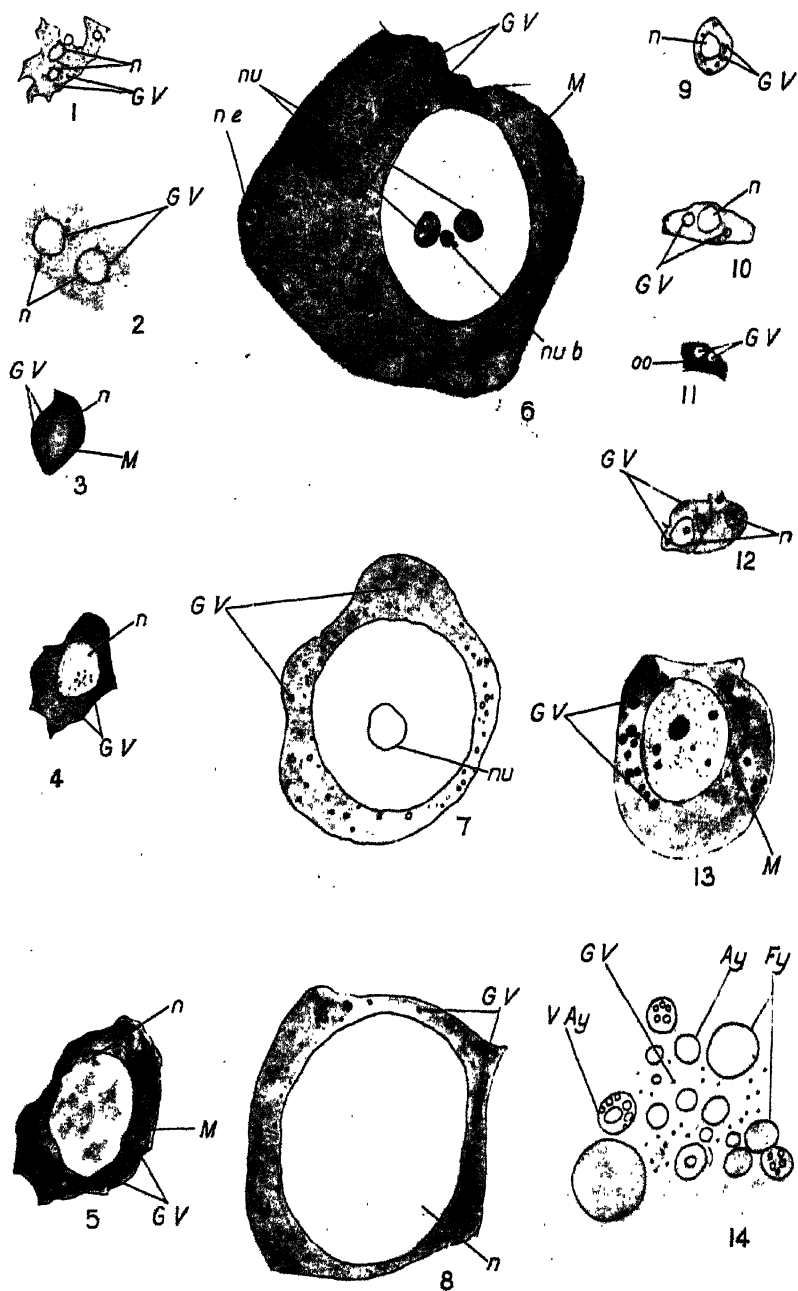
Fig. 21.—Oocyte before yolk-formation showing basophil nucleolus and oxyphil buds.

Fig. 22.—Early stages of yolk-formation. Mann-Kopsch.

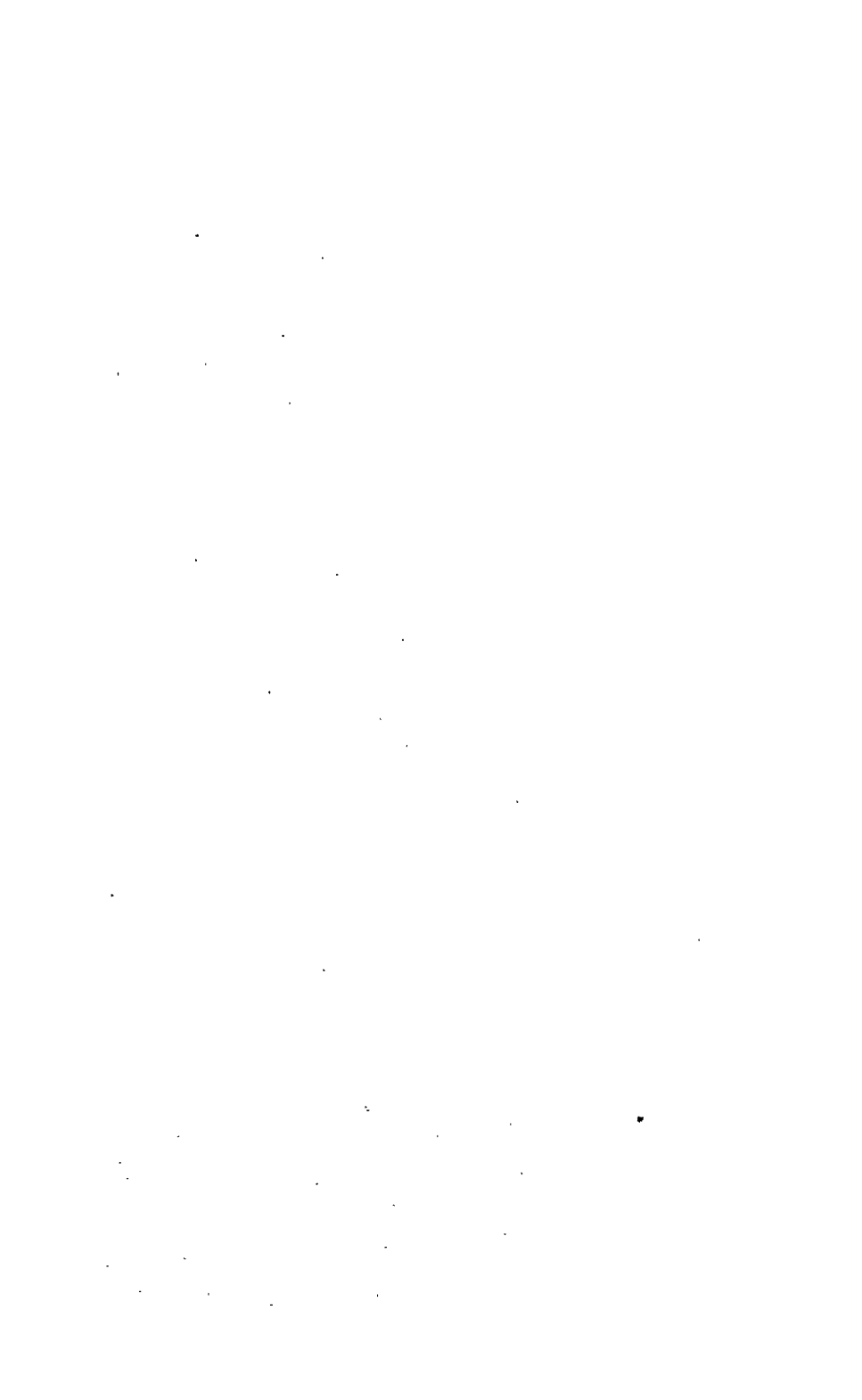
Fig. 23.—Later stage of yolk-formation, showing vacuolated albuminous yolk. Mann-Kopsch.

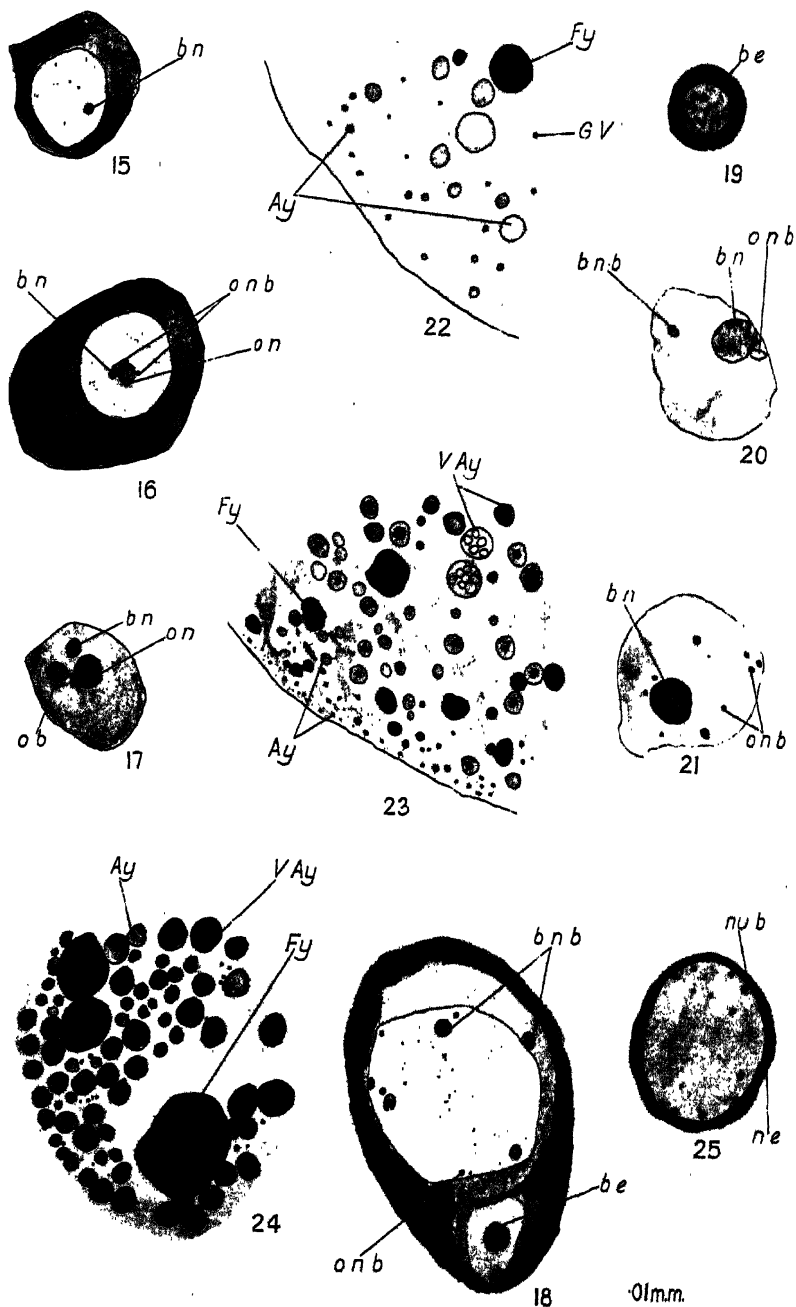
Fig. 24.—Later stage of yolk-formation. Mann-Kopsch.

Fig. 25.—Nucleolar extrusion outside nuclear membrane. Mann-Kopsch.



0.1mm.





0.1mm.



The Neuro-muscular mechanism of the gill of *Pecten*.

By

S. B. Setna, M.Sc.

Zoological Laboratory, Cambridge ; and Royal Institute of Science,
Bombay.

With Plates 16-18 and 4 Text-figures.

CONTENTS.

	PAGE
A. PHYSIOLOGY	366
(a) Responses of the Gill in situ	366
(b) Responses of Excised Gill	368
(c) Responses of Gill to Mechanical Stimulation	370
(d) Responses of Gill to Chemical Stimulation	373
B. HISTOLOGY	374
(a) Ctenidial Axis	375
(b) Gill Filaments	375
(c) Branchial Musculature	377
(d) Nerve Supply	380
i. Palps	380
ii. Gills	380
(e) Osphradium	383
C. GILL MOVEMENTS AND FEEDING HABITS	386
D. SUMMARY	387
BIBLIOGRAPHY	389
EXPLANATION OF PLATES	390

THE gills of *Pecten* have been the subject of much study, their structure being particularly described by Ridgewood (1908), Kellogg (1915), and Dakin (1909). However, there are structures of profound physiological importance present in the gills which apparently have so far remained unnoticed, while data are lacking concerning the neuromuscular mechanism which leads to a definite and orderly distribution of food

material on the gill surfaces. Further, there are only casual references to the sensory reactions of the gill, without which an adequate understanding of their function is impossible. My own observations disagree with those of Kellogg (1915), who writes, 'Extensive movements of the gills of *Yoldia* have been described by Drew (1899) and the writer (1890) in which organs there are well-developed muscles, but in the *Pecten* gill and others also capable of extensive movements such muscles are absent.'

A preliminary survey of the living gill suggested the existence of a neuromuscular mechanism more complex than is indicated from known histological structure. The present work constitutes an attempt to analyse the behaviour of the gill and to correlate this with its histology.

The problem was suggested to me by Mr. Gray. The earlier part of the work was carried out under him and later under Professor Gardiner. To both these gentlemen are due my acknowledgements for their kindly aid and criticisms, and I wish to express my thanks to the University for allowing me to occupy their table at the Marine Biological Station at Plymouth during part of a summer. My thanks are also due to Dr. Allen (Director), Dr. Orton, and other members of the staff for their courtesies to me.

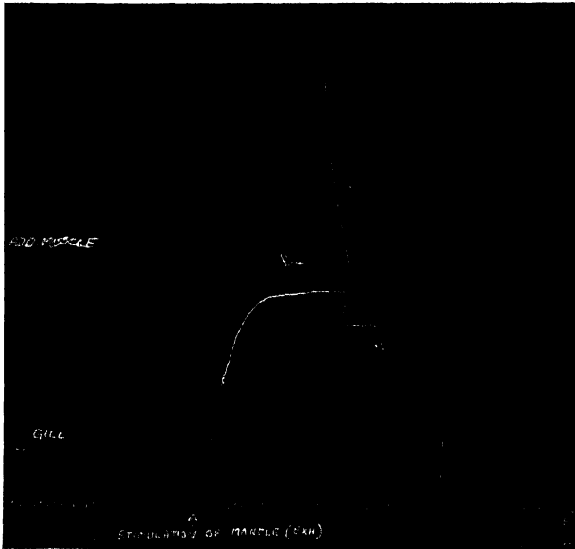
A. PHYSIOLOGY.

(a) Responses of the Gill in situ.

Pecten exhibits a very simple type of reflex activity, the animal responding to all forms of stimulation by the snapping of its shell valves. The response is purposeful, often resulting in the ejection of large quantities of food matter and also of waste material. If the branchial nerve or the palial nerve of the mantle is stimulated, the gills respond by shortening their long axis; they are drawn forwards by the contraction of the ctenidial muscle. When the stimulation is vigorous, the immediate diminution in the extent of the gill is about one-third its original length; the foot at the same time is retracted and only subsequently does the adductor-muscle contract.

This sequence of events is observed by removing one of the shell valves, leaving the animal under water and observing the gills and the foot. After the branchial-adductor cycle, recovery is immediate; the adductor-muscle relaxes first, followed immediately by the relaxation of the gill-muscles.

TEXT-FIG. 1.



Record of response to gentle stimulation on the mantle, resulting in the branchial-adductor cycle. For explanation see text.

A kymographic record of the movements of the gill and adductor-muscle is given (Text-fig. 1). The lower curve represents the gill, the upper the adductor.

The whole cycle can be induced by stimulating either the branchial nerve or the sensory nerves of the mantle (via the palial network).

There is, on the mantle, a well-organized arrangement of excitability; the regions which are particularly sensitive are the anterior and posterior regions of the mantle, where a gentle stimulation calls forth the branchial-adductor cycle; the faeces

and food particles that fall on the mantle are carried by the ciliary tracts on the mantle to the anterior and posterior sensitive regions, where they furnish the mechanical stimulation for this response. If the main branchial nerve is cut (Text-fig. 3 x), the adductor cycle can be induced, but there is no movement of the gills.

This response of the animal is a very specialized feature, and is an extremely important factor for the protection of the gills. This is manifest in the arrangement of the muscular and nervous tissues, and is a necessary consequence of the functional activity of these elements.

(b) Responses of the Excised Gill.

Data in connexion with the sensory physiology of the gills of *Pecten* are extremely meagre, whereas their functions can only be understood by a thorough analysis of the various movements and sensory reactions of both the gills and the palps, and the relation of these important properties to their histological structure.

The gills of *Pecten maximus* exhibit incessant contractions which seem co-ordinated in the case of the demibranchs on each side. The extreme sensitiveness of the gills is remarkable, for these structures are capable of responding not only independently of the brain and visceral ganglia, but also when removed from and quite independent of the body. When they are separated from the body they continue to exhibit great sensitivity and respond for two or three days after their removal; they resemble, in this respect, the tentacles of Actinians (Parker, 1917), which when separated from the body of the parent continue to carry out their various activities.

If the gill is cut vertically along its length into several pieces and left under a current of sea-water, each piece continues to respond like the entire gill in all details just as under normal circumstances; all the different movements being reproduced by the excised pieces. This fact suggests that the highly co-ordinated movements of each ctenidium or each piece of gill

depends on a local mechanism and does not require the interaction of the centralized nervous system. Such activity is exhibited either by muscles which act independently, or under the influence of localized nervous mechanism. In the Pecten gill it is almost certainly due to the latter cause, in view of the intimate relationship of muscular and nervous tissue. Since Englemann (1869) published his work on the Rabbit's ureter, the phenomenon of rhythmical spontaneous movement has been observed in a large number of smooth muscle preparations. Contraction of smooth muscle by direct stimulation is quite common. Lewis and Lewis (1917) demonstrated this in the amnion of the chick embryo. The smooth muscle-cells undergo contraction as early as the fourth day, when there is no nerve supply whatever.

Burrows (1912) showed that very small pieces of heart-muscle from embryos of the chick continued to beat in blood-plasma for as long as thirty days, and the cells in this mass divided and separated from the rest of the tissue and beat rhythmically. In the case of the vertebrate heart, it has been recognized that though the heart-beat is inhibited and accelerated by nerve impulses, the maintenance of its rhythm is independent of its nerves.

The movements of the Pecten gill persist for several hours even after they have been separated from the body and cut vertically into pieces. Such autonomous organs are generally regarded as possessing a nerve-net, that is, a tissue in which there is protoplasmic continuity between the nerve-fibres and nerve-cells; this property is associated with the power to conduct impulses in all directions. Bethe (1908), who investigated *Rhizostoma*, described elongated ganglion-cells with several nerve-fibres which anastomose with the processes of neighbouring cells. Parker writes: 'As a result of the intimate relation usually existing between the nerve-net and the muscles that it controls, most organs that are provided with this type of neuromuscular organization exhibit an extreme degree of autonomy. This is perhaps one of the most striking features associated with the nerve-net. It is well illustrated by such an organ as the

tentacle of the sea anemone, the autonomy of which was long ago recognized by von Heider (1897).'

Another portion of the vertebrate body that exhibits autonomy and at the same time possesses a nerve-net is the digestive tube, especially the small intestine, between the two muscular coats of which is a network of nerve-fibrils, i.e. the plexus of Auerbach. If a portion of the gut-wall is separated into two parts, that portion which includes the myenteric plexus only will respond.

(c) Response of the Gills to Mechanical Stimulation.

1. Concertina action of gill lamellae following mechanical stimulation.

If the gill is stimulated mechanically either by touch or by the presence of a few particles of carmine, two very distinct reactions follow.

(a) If the frontal surface of the gill is lightly touched with a needle, or if a few particles of carmine are placed on its surface, the two lamellae move towards each other (Text-fig. 2). I shall term this the shutting response. The extent of this reaction is apparently proportional to the strength of the stimulus. If the latter is strong the response is instantaneous, the lamellae moving towards each other very actively, while the contraction extends to the filaments in both directions along the gill. If the stimulus is weak only the filaments directly affected are involved.

(b) If a pinch of carmine particles is placed on a lamella, a variation of response occurs. The lamellae instead of moving towards one another first separate slightly further, so that the positive reaction changes to negative; evidently in this case the muscles are differently excited to produce this reaction. This separation of the lamellae is referred to as the opening response, and both the opening and shutting responses which go on quite regularly in nature as a result of stimulation by food particles is described as the 'concertina action'.

A point of special importance is the fact that it is usually the

lamella which is not touched which moves towards the one stimulated. This type of response calls to mind the responses of the siphons (Hecht, 1918), where stimulation of the inside of one siphon results in the closure of the other, the stimulated siphon remaining wide open while the sphincter of the other siphon is called into play (crossed responses).

The most sensitive parts of the gills are those which correspond to the distribution of the main nerve-trunk, i. e. the branchial nerve. The response of the gill to mechanical stimulation is not

TEXT-FIG. 2.



Diagram showing the behaviour of lamellae on being mechanically stimulated with carmine particles, shown as minute dots.

impaired by severing the connexions with the brain or by cutting the gill vertically into pieces, but if the branchial nerve is cut away, stimulation fails to call forth the 'concertina action'. This is further seen in the extreme anterior portion of the gill which is devoid of the main branchial nerve and is the least excitable portion. When the gill is divided into two parts by a cut at Y (Text-fig. 3), the part A shows no 'concertina action' on stimulation, whereas the part B shows these movements as before.

2. Flapping action of the individual principal filaments.

Besides the 'concertina action' described above, the gill lamellae show a curious flapping action which is not dependent on the main nerve-trunk; excision of the main branchial nerves does not bring about a cessation of this response. In such cases the 'concertina action' ceases.

The flapping movements are due entirely to the principal filaments. Isolated principal filaments when examined under the binocular microscope, being gently touched with a fine glass tube, respond by twisting themselves and moving their sides up and down at right angles to their length like the flapping movements of a bird's wing. The movement is powerful,

characteristic, and the response immediate; the ciliary discs are the most active regions and if to the principal filament are attached any ordinary filaments they also are carried up and down mechanically. When ordinary filaments, cut away from the principal filaments, are stimulated, there is no response. It is therefore clear that the movement of these intervening filaments in the entire gill is mechanical, as the removal of principal filaments render the ordinary filaments motionless.

It matters little whether the stimulus is applied at the ends, in the middle, or near the sides, the response is immediate and the duration of the response extends for several seconds beyond that of the stimulus. Such isolated principal filaments continue to show activity for several hours and are capable of being stimulated by agitation and by drops of water which are allowed to fall upon them. In an attached gill very weak stimuli will call forth this response. If the stimulus is slightly stronger the 'concertina action' comes into play.

I have already mentioned that severing the main branchial nerve produces no appreciable effect on the excitability of the principal filaments; this means that, like the tentacles of *Metridium*, they have within their structure a nervous mechanism for an unusual degree of autonomy.

In the principal filaments this characteristic is dependent on the presence of nerve-cells on either side of the chitinous tube (n, fig. 2, Pl. 16). On the application of methylene-blue these cells present a picture that looks very much like a nerve-net; nothing, however, can definitely be said regarding the relation of these to one another, because in my very rare successful preparations the methylene-blue did not sufficiently differentiate the various elements as to render the form and arrangement of the cells obvious; moreover, such preparations can be watched only for a few minutes, and it is not possible to say whether the cells with their branches form an intimate anastomosis like that figured by Bethe (1903). Another difficulty in such cases is to decide which of the processes arising from a nerve-cell is the axone. There is in connexion with the stratum of nerve-cells on the gill-axis, a felt of very fine fibres which can easily be

followed (fig. 3, Pl. 16); these, in some cases, are very closely applied to the axones of the bipolar and multipolar cells. In such cases it becomes impossible to decide whether there is protoplasmic continuity or merely physical contact. Direct tests of the transmission of nervous impulses could be carried out, because of the form and structural modifications of the principal filaments. In the functioning gill the movement of the filaments vary in the different parts of its length. In some regions the gill concertinas its lamellae, whilst in others it shows flapping movements of its principal filaments. The full movements appear irregular and quite devoid of rhythm, being in reality dependent on the nature and size and on the position of the stimulating bodies either naturally carried into the mantle chambers or artificially set on the gills during the experiments.

Different species of *Pecten* manifest different degrees of sensitiveness to touch stimuli which in the case of *P. maximus* is exceedingly high and in *P. tigrinus* is very low.

(d) Responses of the Gill to Chemical Stimulation.

Dakin (1910) performed experiments on *Pecten* with osphradium destroyed; but, as the extent of the structure was not then well known, and since in his experiments he failed to remove the entire organs, it is possible that the responses he observed misled him in his interpretation. Experiments of very simple nature were performed by me to test the sensitiveness of the gills with a number of substances, such as alcohol, clove-oil, picric acid, hydrochloric acid. Weak solutions of these were discharged through a fine capillary pipette into the various regions of the gills. On the application of these in the region of the osphradial ridge there was a slight mechanical disturbance; other parts of the gills are very much less sensitive to relatively stronger solutions. Sea-water and star-fish extract discharged into the various regions did not induce any noticeable response. It is evident from these experiments that the gill is sensitive to chemical stimulation and by means of the osphradium is capable of detecting slight changes in the surrounding medium, or, like the olfactory organs of the fish, serving to test

the chemical nature of the water that passes over it, relates the organism to its environment.

It might be mentioned that the continuation of the osphradium on the gill-axis on each side seems anatomically to be well adapted to receive such stimuli because the gills are the first structures which come into direct contact with the water-current flowing either through the gills or over them.

B. HISTOLOGY.

The reactions of the gill, described above, lead to the conclusion that there must exist nervous and muscular elements which have escaped the notice of previous observers. The behaviour of the living gill points to the presence of sensory cells on the ctenidial axis and on the frontal surface of the gill. These elements must be associated with muscular elements responsible for the movement of the gills themselves, and at the same time must be associated with the mechanism responsible for the adductor-muscle cycle. In addition to this localized branchial complex, there must be a nervous connexion between the mantle and the gills whereby stimulation of the former induces a contraction of the ctenidial axis.

The following species were examined histologically: *P. maximus*, *P. opercularis*, and *P. tigrinus*. Most of the histological and experimental observations were confined to the first species on account of its large size.

In working out the histological details many different methods were employed, but no particular method could be singled out as being the best; many were entirely unsuccessful.

Most of the well-known fixatives were employed and after a trial were discarded in favour of a modification of Bouin's (Duboseq) fluid which was made up as follows:

Sat. sol. picric acid in 70 per cent. alcohol .	2 parts.
Sat. sol. corrosive sublimate . . .	1 part.
40 per cent. formalin . . .	1 part.
Glacial acetic acid . . .	1 part.

The well-known silver and gold methods were also tried in

an attempt to demonstrate the finer fibrillar endings, but the results were not good.

(a) The Ctenidial Axis.

The ctenidial axis is clearly built up of muscular and connective tissue; on the outside is the usual epidermis formed of a single layer of cells. In this layer sensory cells occur in very large numbers, the cells differing from the ordinary epithelial cells in that they are slightly larger and possess a bundle of fine cilia much longer than the cilia of the ordinary epithelium. These sensory cells, at their deeper ends, are connected with nerve-fibres in the nervous layer which is well differentiated below the epithelium on both sides of the axis. In this layer are found scattered at frequent intervals large branching nerve-cells (fig. 3, Pl. 16) which are spindle-shaped, bipolar, and multipolar; the nerve elements are clearly differentiated from the other tissues by the very large size of their cell-bodies and by their large nuclei. Beneath the epidermis the nerve-fibres can readily be followed in frozen sections stained by the iron haematoxylin method; the fibres are fairly distinct and run for some distance and seem to anastomose in places. The different methods of methylene-blue adopted proved more or less unsatisfactory. Nissl's-blue stained the nerve-cells fairly well and also the fibres. The presence of a nerve-plexus and of the stratum of ganglion-cells is clearly established.

Fragments of ctenidial axis, like pieces of excised gill, may be kept alive for several hours, and when stimulated continue to show slight muscular contractions and twisting. It would appear that when the sensory cells are stimulated, the impulse is transmitted to the ganglion-cells which, as seen later, I have reason to believe directly excite the muscle to action. The observed sensitivity of the ctenidial axis to direct stimulation is thus directly associated with the presence of a localized receptor mechanism.

(b) The Gill Filaments.

As is well known, the principal filaments are connected to their neighbours along the lamellae by interfilamentar junctions, as

also are the ordinary filaments with one another; these junctions in the three species described take the form of discs provided with stiff cilia (*c.d.*, fig. 2, Pl. 16). Besides these ciliated discs, especially in *P. opercularis*, the ordinary filaments on either side of the principal filaments possess a ridge appearing in section as a spur turned towards the principal filaments. The principal filaments themselves possess lateral extensions (figs. 5, 6, and 7, Pl. 17), so that the whole system forms accessory interlocking arrangement and may be looked upon as an interesting mechanical device by means of which the flapping extensions on the principal filaments fit into the groove formed by the spur. As already pointed out, the principal filaments are extremely active and are responsible for what is described as the 'flapping' movement. It is therefore reasonable to expect in this region of great activity an additional device which, when the very active principal filaments are moving up and down, causes the extensions to slip into the groove and so prevents detachment of the filaments. The same mechanism also transmits movement to the inert ordinary filaments.

Lining the chitinous tube of each gill filament there is a layer of endothelial cells (*e.*). The previous accounts of the histology, so far as they relate to this layer, reveal a number of contradictory statements and opinions. Its presence has been recorded by a number of authors in different Lamellibranchs. Thus Kellogg noted a definite lining in *Pecten* and also in a number of forms, whereas Pelseneer, Janssens, Ridewood, and Dakin deny the presence of a continuous endothelium.

My material fixed with Bouin's fluid and stained with iron haematoxylin, or Dobell's methyl-blue-eosin, shows very clearly the endothelial lining as a definite and continuous layer in all the species of *Pecten*. With methyl-blue-eosin the chitin stains blue and the endothelial lining, as well as the intra-filamentar septum red, and the nuclei of the endothelium stand out distinctly from the red corpuscles.

In connexion with the principal filaments are developed the highly vascularized respiratory expansions (*r.e.p.*, Text-fig. 4 and fig. 7, Pl. 17) to facilitate the absorption of oxygen by the

blood-stream. For a detailed description of these structures the reader is referred to Dakin's monograph.

(c) Branchial Musculature.

The histology of the muscular system in molluscs has received considerable attention; reference to the literature shows that opinion is divided with regard to the presence of cross striation of fibres. Von Ihering (1878) and Kellogg (1890, 1892) record the presence of cross striation in the fibres of adductor-muscles. Fol (1888) and Roule (1888) deny the existence of striated muscle-fibres, and, according to them, only smooth muscle-fibres are bound in the molluscs. Dakin (1909) drew attention to the striped muscles in the mantle edge of *P. jacobaeus* and *P. opercularis*, where there are transverse striations as in the adductor-muscle of *Pecten*. No reference is made to the muscle-fibres that move the filaments. It is indeed stated by Kellogg that in the gill of *Pecten*, and of other Lamellibranchs also capable of extensive movements, muscles are absent. Dakin draws attention to the interesting fact that mantle-muscles which show obvious striations are engaged in rapid movement, which is also organized and related to the closing and opening of the shell in swimming. To these may be added the longitudinal ctenidial muscles (Text-fig. 4, *l.c.m.*) as another case of connexion between striation and rapid contraction and relaxation, but, strange to say, such striated fibres were found only in *P. tigrinus* and *P. opercularis*, not occurring so far as I could ascertain in the ctenidial muscles of *P. maximus*.

Besides these muscles there are in relation to the filaments two other sets which bring about rapid movement and these belong to the smooth muscle type.

The difficulty in distinguishing muscles from connective is great, but the functional relation and microchemical reactions leave no doubt as to their muscular nature. The arrangement of the two sets can be best understood by a reference to Text-fig. 4 and fig. 9, Pl. 18, and the fibres shall be referred to as the transverse (*t.m.f.*) and the criss-cross set (*c.c.m.*).

The transverse set runs transversely across the filaments, and its fibres are inserted on the dorsal and outer sides into the chitin of the principal filaments.

The criss-cross set lies below the filaments, and is also inserted into the chitin of the principal filaments; but lower down to the inner sides, as their name indicates, they run in a criss-cross manner.

The lamellae respond by moving towards each other; these movements characterize just such a group as those described later, and such a function is closely indicated by the direction of the fibres and their relation to the chitinous filaments. These muscles can easily be seen in sections, but the reconstruction of the course they take is a difficult matter, because during fixation the lamellae undergo some amount of distortion.

The functional relation is somewhat as follows. The lower criss-cross fibres by their contraction draw the filaments together; in such a condition the dorsally placed fibres are stretched. On the relaxation of the inner set, the mechanical contraction of the transverse muscles causes the lamellae to separate. It is possible to remove the criss-cross muscle-fibres; when this is done with a red-hot needle, the characteristic response disappears and the lamellae begin to gape. These fibres are usually single, and the individual fibres are large. They have a definite arrangement and present a wavy appearance. The contours and forms of the individual fibres as they appear under the microscope suggest both smooth muscle and connective tissue-fibres.

A number of stains were employed to determine the real nature of the fibres. The material used was fixed in corrosive sublimate.

1. Mallory's connective tissue stain.

The white fibres and reticulum of connective tissue stain blue and the elastic fibres yellow in this medium.

Paraffin sections fixed in corrosive sublimate were stained for about ten minutes in a $\frac{1}{2}$ per cent. aqueous solution of acid fuchsin, rinsed in distilled water and treated for two minutes in 1 per cent. solution of phospho-molybdic acid; they were again

rinsed in distilled water and transferred to the stain for about fifteen minutes, after which they were rapidly dehydrated, cleared, and mounted. After such treatment the white fibrous connective tissue stained light blue and the two sets of fibres in question stained deep red. This would indicate that the fibres are not composed of white fibrous tissue.

The presence of elastic fibres was sought by means of Weigert resorcin-fuchsin stain, in which the elastic fibres should stain dark blue.

Sections were stained overnight in the above mixture. They were then dehydrated quickly, cleared in xylol and mounted. The connective tissue in the ctenidial axis and round the muscle-fibres took up a dark-blue coloration, whereas the fibres in question appeared dull lavender-grey, and did not show any affinity for the stain. This would mean that the fibres are not composed of elastic connective tissue. The control in all these cases were the ctenidial muscles.

Van Gieson's picro-nigrosin stains muscles yellowish green and connective tissue blue. Sections were therefore stained by this method for twelve hours, then washed in picric alcohol, rapidly dehydrated, cleared, and mounted. The connective tissue stained blue, the fibres and the ctenidial muscles green. The fact that the fibres in the above experiments react in precisely the same manner as the ctenidial muscles when subjected to the treatment of different stains show that they are themselves composed of muscle-fibres.

Van Gieson's picro-fuchsin stains muscles yellow and connective tissue red. Sections stained in this medium gave the above reaction.

From these reactions and from those already described regarding the functional relation of the fibres, there are definite grounds for believing that the fibres are composed of muscle-tissue. In addition to these muscles there are other muscle-fibres which run into the interfilamentar junctions (*m.f.*, fig. 2, Pl. 16) and also throughout the length of the principal filaments, and it is due to these that the principal filaments exhibit activity.

(d) Nerve Supply.

(i) Palps.—The palps were shown to be innervated by a branch from the cerebral ganglion (Drew, Dakin), but the definite course of this nerve was not worked out, and the arrangement and connexions of nerve-trunks in the region of the mouth proved to be of exceptional interest.

From the anterior end of the cerebro-pleural ganglia arise four nerve-cords. Two of these are large and well developed. The inner one (*c.p.c.*, fig. 1, Pl. 16) is the commissure that joins the cerebral ganglia; while the other well-developed cord arising from the anterior pleural end is the anterior palial nerve (*a.p.n.*). Between these two trunks arises a small nerve which runs parallel with the commissure as far as the mouth region, where it joins an enlargement which also receives branches both from the dorsal and ventral edges of the mouth. From this enlarged region extends a fairly stout nerve, consisting of nerve-cells and fibres so that nerve-cells are equally distributed along the whole length; this runs just below the junction of the two palps on either side; it sends out branches to the palps and near the anterior neighbourhood of the gills it splits up into several smaller branches, some of which are continued into the gills. The fourth small nerve takes its origin from the pleural end, runs on the outer side of the anterior palial nerve and ends as shown. In one individual an interesting variation occurs: the palp-nerve which arises between the two main nerve-trunks actually branches off from the small outermost nerve near the middle of its length; the two nerves, figured distinctly, are both referred to as palp-nerves (*n.p.*).

(ii) Gills.—In the gills besides the scattered nerve-cells and the anastomosis of their fibres over the whole organ, there are four main longitudinal trunks (Text-figs. 3 and 4).

The main branchial nerve arises from the visceral ganglia, bends ventrally and posteriorly and enters the ctenidial axis, each nerve supplying the gill of its own side.

1. The branchial nerve gives origin at intervals corresponding to the principal filaments throughout its length to one single

and two pairs of nerves. The single nerve innervates the osphradium. The succeeding pair of nerves run laterally and are continued down the blood-channels on the principal filaments (*n.f.*, fig. 8, Pl. 17). The last pair of nerves run between the ctenidial muscles and outer epithelium and enters into

TEXT-FIG. 3.

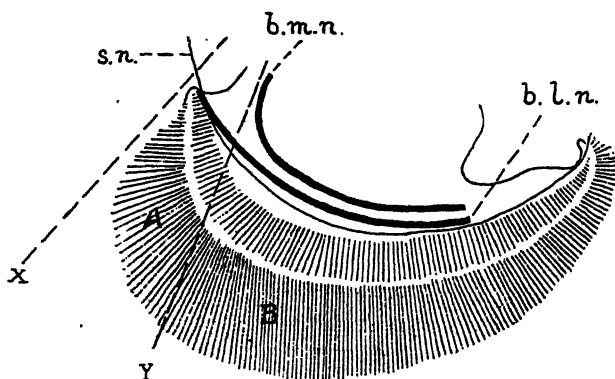


Diagram showing a side view of the component parts of the gill.

anastomosis with groups of ganglion-cells which represent the two lateral nerve-trunks.

2. The two lateral nerve-trunks (*b.l.n.*) run parallel to the main branchial nerve, and consist almost entirely of groups of ganglion-cells; these nerve-trunks are referred to as two large ganglia. Fibres from these are continued down on each side, they turn inwards below the ctenidial muscles and supply the principal filaments (*n.f.*). Some fibres from these ganglia also seem to enter the ordinary filaments.

The fourth nerve-trunk which arises from the brain is the subsidiary branchial nerve (*s.n.*). It runs the entire length of the gill and lies below the main nerve. In frozen sections stained by the Bielschowsky's method, fibres from this nerve to the transverse muscle are easily distinguished (*s.n.f.*, fig. 9, Pl. 18). By reason of the presence of nerve-fibres, in sections of ordinary filaments, considerably anterior to the three longitudinal nerves

described above (Text-fig. 2), it seems probable that the subsidiary nerve innervates these filaments as well. The supply from this nerve is scanty as compared with the extensive innervation from the main nerve-trunk.

All attempts to determine the function of the subsidiary branchial nerve yielded, unfortunately, negative results. Of these, the following may be of interest.

1. Immediately the shell was removed and while the gill lamellae were together (that being their usual condition before shell removal), a cut was made at *x* (Text-fig. 2) to see what influence the nerve has on the effector system of the gill. On gentle stimulation the lamellae moved apart as rapidly as in the case of the other gill in which the connexions were not broken, neither were the spontaneous contractions which sometimes occur modified in any appreciable manner.

2. From one animal a gill was removed from the body and a comparison of its behaviour with that of the intact gill was made. Both the gills responded to stimulation in a similar manner; they opened and closed in the same amount of time for the first half-hour or so, later the same method of treatment caused the excised gill to open much quicker than the other. The experiment was repeated again on fresh material, but I was not able to obtain any very conclusive evidence for the existence of inhibitory activity.

An attempt was also made to stimulate the nerve in the middle of its length and also near the palps; varying strengths of current were used, but in no case had this any observable effect. While its sensory function cannot be denied, another possibility is that the cilia on the palps and the gills may be under nervous control.

Nelson (1924) in spat oysters describes the rejection of particles as due to reflex erection of the ridges of the palps which bring into action groups of cilia which beat away from the mouth.

On cutting the subsidiary branchial nerve, however, there is no evidence of reversal either on the gills or on the palps, nor does mechanical stimulation alter the direction of the ciliary

stroke. The function of the subsidiary branchial nerve remains, therefore, obscure.

(e) The Osphradium.

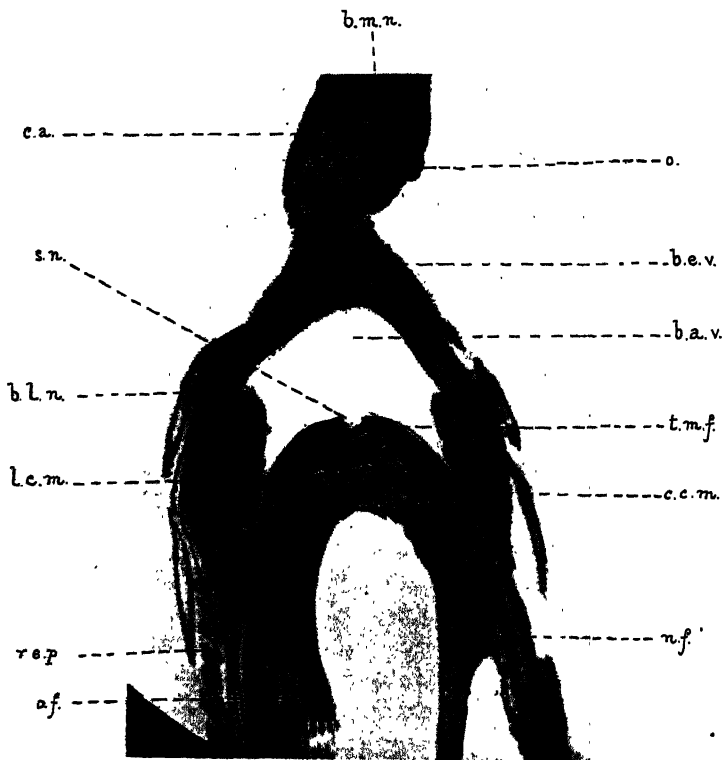
A reference to standard literature is sufficient to show that the organ has been extensively studied since its discovery in 1881 by Spengel. It has been the subject of a paper by Dakin (1910), who described the anatomy and considered the structure in a comparative way in a number of Lamellibranchs with a review of the question of innervation. His description of the structure and innervation of the organ as present in the vicinity of the visceral ganglion is in accordance with my observations.

As to the function he writes: 'The experiments made with the osphradia removed were unfortunately not quite conclusive enough. These organs, as described above, lie in close proximity to the visceral ganglia. An attempt was made to remove the sensory epithelium by scraping with a scalpel; however, since the osphradia are unpigmented, it was not possible to see whether any remains of these organs were left. The individuals, however, treated in this way appeared to react to the star-fish pulp, &c., &c. On the whole, I believe, the star-fish pulp was recognized by the mantle edge with its sensory tentacles and any action of the osphradium is very questionable.'

In my sections of the gill axis, the most interesting feature is a continuation of the osphradium in the form of a ridge on the gill axis (Text-fig. 4, o.). Most of the fixatives that I employed brought about shrinkage, distortion, and destruction, as a result, to the naked eye, the osphradium is scarcely visible, but there is never any difficulty in recognizing it under the microscope, as it is easily identified by its elevated nature, in stained or unstained sections. It is a constant organ of the gill axis and for the reasons mentioned above has remained unrecognized so far. I examined this organ by the help of several sets of serial sections in different planes, the osphradium is seen to be continued on either side of the gill axis to the extreme tip of either gill. It occurs in the position described in all the species of *Pecten* investigated by me, as well as in *Glycimeris*

glycimeris. It takes a course parallel to the main branchial nerve; sometimes on a level with it and at others a little below. It is innervated from the main branchial nerve, bundles of delicate nerve-fibres are given off on its side; these arise at

TEXT-FIG. 4.



Photomicrograph of a transverse section of a portion of gill showing the arrangement and relation of its different parts.

intervals and enter the structure at its base (*b.n.f.*, figs. 9 and 10, Pl. 18).

The epithelial cells of the region of the osphradium are more or less columnar and consist of central cells which are somewhat larger than the surrounding cells. They are broader in the

middle, in which portion the nucleus is situated ; the basal end is the narrowest region and seems to be continued into a nerve-fibre. At their anterior ends the cells appear to be prolonged into stiff cilia or sensory hairs, which lie below the well-developed cuticle. Such cilia, and to some degree the total structure, can be seen in some regions, but was not demonstrable as a regular feature along the whole length of the organ. List (1902) defines the osphradial area as follows : ' The cells of the osphradial area are to be distinguished by a complete absence of cilia, a distinct marginal cuticle, and an increased depth.' In *Pecten*, according to my observations, only the last two characteristics are present ; there are in addition to the structures described above well-developed sensory cells with their cilia in the osphradial area (*s.c.*, fig. 10, Pl. 18).

Another feature of the osphradium in this position is the presence of bipolar cells. Besides the bundle of nerve-fibres from the main branchial nerve, the osphradium has another bundle of fibres running in the middle throughout its course ; these fibres (*l.n.f.*, figs. 10, 11, Pl. 18) run below the epithelium parallel to the main branchial nerve-trunk, amongst its fibres bipolar cells occur (*b.c.*, fig. 11, Pl. 18). Dakin gives a detailed description of the innervation of this organ. It is not my object to repeat Dakin's observations, but an attempt was made to trace to its origin the longitudinal bundle of nerve-fibres without any success ; this was not determinable either by dissections or in sections, but they must come either from the brain or from the visceral ganglia.

In this connexion it might be interesting to mention Dakin's view : ' It is quite possible and probable that processes of some of the ganglion-cells concerned in the innervation of the osphradium pass into the cerebral and visceral connectives in addition to those which enter the visceral ganglion, but it is not easy to follow individual fibres the necessary distance in sections, and the evidence appears to show that the visceral ganglia are concerned in the innervation of the osphradium.'

C. GILL MOVEMENTS AND FEEDING HABITS.

As is well known, there are several ciliary currents on the surface of the gill.

1. A very fast frontal system which runs up the grooves on the principal filaments, along the top edge of the gill, between the palps, to the mouth.

2. A system of slower frontal currents which run down the crests on the ordinary filaments, along the free edge of the gill into the groove at the edge, where the particles are formed into slimy strings; these usually find their way on to the mantle from whence the accumulation is thrown out by the branchial adductor-cycle.

Carmine particles dropped on the gills are thus at once sorted out into two definite streams, one moving towards the base and the other towards the edge. These directions of movement in agreement with the ciliary activity are not always constant, since certain of the larger particles may leave their course, be moved on to the crests and continue their course in the opposite direction. This act of transference being evoked by the presence of the large or more irritating particles on the principal filaments, the impulses due to their presence are transmitted up and down the principal filaments and the flapping movement, which is a very adaptative response, results. It is the most useful movement to get rid of the large particles, and this it evidently accomplishes by throwing them on to the crests of the adjoining filaments; this activity results in a continuous arranging and rearranging of particles. The fine particles, which are more firmly held in the mucus on the principal filaments, continue their course unaffected; whether these would find their way into the mouth depends largely on the activities of the palps.

Finally, under the influence of strong stimuli, the flapping movement is followed by vigorous concertina action; this striking sensory response, resulting in proportionally greater activity, deals with the bulk of material and its primary importance is that it serves as an expelling force.

The downward moving streams of food collect in the grooves

at the edge of the gill and are propelled anteriorly. When the accumulations in this position become fairly large, the gills twitch owing to the mechanical pull of mucus threads, so that the load is dislodged on to the mantle. If the string of mucus by any means persists in sticking to the edge, the response is repeated and a concerted effort seems to be made by the neighbouring filaments which bend in the direction of the stimulating body and try to dislodge it. For a more complete account of the ciliary currents on the gill, reference should be made to Kellogg's paper.

The data presented in the present paper seems to justify the view that the selective feeding mechanism of the gills of *Pecten* is not solely the result of ciliary activity, but is the result of a highly co-ordinated system in which ciliary, muscular, and nervous elements all play an important role.

The experimental and histological data are clearly in harmony with each other, although no function is as yet assignable to the subsidiary branchial nerve, nor is it possible to demonstrate an exact parallel between the distribution of the diffuse receptor-cells on the gill-surface and the areas which respond to mechanical and other forms of stimulation. On the other hand, each physiological phenomenon has been traced to specific histological elements—and an improvement of technique may, in the future, result in still closer approximation of structural form to physiological activity.

D. SUMMARY.

Experimental.

1. The contraction of the adductor-muscle which follows stimulation of the palial nerve is preceded by a marked contraction of the ctenidial axis, so that the gill contracts before the adductor-muscle becomes active. This movement of the ctenidium is abolished if the main branchial nerve is cut near its origin.

2. The gills of *Pecten* possess a neuromuscular mechanism which is to some extent independent of the rest of the body; so that excised gills when stimulated react in the same way as an attached gill.

3. The lamellae of the gill possess two distinct types of movement.

- (a) When the surface of the gill is stimulated by contact with a glass rod or by carmine particles, the frontal surfaces of the two lamellae approach each other ; the movement very often being executed by the lamella which is not actually being stimulated. The lateral extent of these movements (concertina movements) is roughly proportional to the intensity of the stimulus. Such movements normally appear to transfer the bulk of the material on to the mantle. Separation of the main branchial nerve abolishes these movements.
- (b) Each principal filament is capable of moving the ordinary filaments to which it is attached. This movement (flapping movement) is due to the movements of the interfilamentar junctions which alternatively move up and down at right angles to their length. This motion is independent of the branchial nerve and can be produced by direct stimulation of very tiny pieces of the individual filaments.

4. The significance of gill movements to feeding habits is discussed. The course of food particles depends on the nature of the stimuli affecting the gill.

Histological.

5. The ctenidial axis and the principal filaments have a stratum of anastomosing nerve-cells which appear to form a true nerve-net comparable to that of the mantle.

6. The gill receives nerve-fibres from two sources, the brain and the visceral ganglion. The subsidiary branchial nerve is a structure hitherto unknown in the molluscan gill ; so far its function is unknown. Each gill has four main longitudinal nerve-trunks.

7. The osphradium of the gill has a much more extensive distribution than has hitherto been supposed.

8. Two sets of muscles exist at the base of the gill-filaments,

and these are responsible for movements of the lamellae. The muscle-fibres are non-striated.

9. The principal filaments are connected to the ordinary filaments by processes containing true muscle-cells, and by these cells movements of the filaments are effected.

LITERATURE.

- Ihering, H. von (1878).—"Ueber Anomia, nebst Bemerkungen zur vergleichenden Anatomie der Muskulatur bei den Muscheln", 'Zeits. Wiss. Zool.', bd. 30.
- Fol, H. (1888).—"Sur la structure microscopique des muscles des Mollusques", 'Compt. Rend.', tome 106.
- Kellogg, J. L. (1890).—"A contribution to our knowledge of the morphology of the lamellibranchiate Molluscs", 'Bull. U.S. Fish. Comm.', no. 10.
- Engelmann, T. W. (1898).—"Cils vibratilis". In C. Richet, 'Dictionnaire de Physiologie', tome 3.
- Drew, G. A. (1899).—"Yoldia limatula", 'Mem. Biol. Lab. Johns Hopkins Univ.', 4.
- Herdman, W. A. (1899).—"Gill of Pearl Oyster", 'Journ. Linn. Soc.—Zool.', vol. 29.
- Bethe, A. (1903).—"Allgemeine Anatomie und Physiologie des Nervensystems". Leipzig.
- Ridewood, W. G. (1903).—"Gills of Lamellibranchs", 'Phil. Trans. Roy. Soc. London', 195.
- Parker, G. H. (1905).—"The reversal of ciliary movements in Metazoans", 'Amer. Journ. Physiol.', vol. 13.
- Drew, G. A. (1906).—"The habits, anatomy, and embryology of the Giant Scallop (*Pecten tenicostatus*, Mighels)", 'The Univ. of Main Studies', vi.
- Dakin, W. J. (1909).—"Pecten", 'L. M. B. C. Memoirs', no. 17.
- (1909).—"Striped muscles in the mantle of the Lamellibranchs", 'Anat. Anz.', bd. 34.
- (1910).—"The visceral ganglion of Pecten with some notes on the physiology of the nervous system and an inquiry into the innervation of the osphradium in the Lamellibranchs", 'Mitt. aus der Zool. Stat. zu Neapel', vol. 20.
- Orton, J. H. (1913).—"On ciliary mechanisms in Brachiopods and some Polychaetes, with a comparison of the ciliary mechanisms on the gills of Molluscs, Protochordata, &c.", 'Journ. Mar. Biol. Assoc.', vol. 10.
- Allen, W. R. (1914).—"The food and feeding habits of Freshwater Mussels", 'Biol. Bull.', vol. 27.
- Kellogg, J. L. (1915).—"Ciliary mechanisms of Lamellibranchs, with Descriptions of Anatomy", 'Journ. Morph.', vol. 26.

- Grave, Caswell (1916).—"The process of feeding in the Oyster", 'Science', New Series, vol. 44.
- Parker, G. H., and Titus, E. G. (1916).—"The structure of *Metridium* (*Actinoloba*) *marginatum* Milne-Edwards, with special reference to its neuromuscular mechanism", 'Journ. Exp. Zool.', vol. 21.
- Parker, G. H. (1917).—"Nervous transmission in the Actinians", *ibid.*, vol. 22.
- (1917).—"Movements of the tentacles in the Actinians", *ibid.*
- (1917).—"The effector systems of Actinians", *ibid.*
- Cobb, P. H. (1918).—"Autonomous responses of the labial palps of *Anodonta*", 'Proc. Nat. Acad. Science Philadelphia', vol. 4.
- Hecht, S. (1918).—"The Physiology of *Ascidia atra*, Lesneur", Sensory Physiology, 'Journ. Exp. Zool.', vol. 28.
- Parker, G. H. (1919).—"The Elementary Nervous System", Philadelphia.
- Allen, W. R. (1921).—"Studies on the Biology of Freshwater Mussels", 'Biol. Bull.' (Woods Hole), vol. 21.
- Churchill, E. P. (1923).—"Food and feeding habits in Freshwater Mussels", 'Bull. U.S. Bur. Fish.', 39.
- Churchill, E. P., and Sara, I. L. (1924).—"Food and feeding habits in Freshwater Mussels", *ibid.*
- Yonge, C. M. (1928).—"Feeding mechanisms in the Invertebrates", 'Biological Reviews', vol. 3, no. 1.

EXPLANATION OF PLATES 16-18.

LIST OF ABBREVIATIONS.

a., axone; *a.f.c.*, abfrontal cilia; *a.p.n.*, anterior palial nerve; *b.*, blood-corpuscles; *b.a.v.*, branchial afferent vessel; *b.c.*, bipolar cell; *b.e.v.*, branchial efferent vessel; *b.l.n.*, branchial lateral nerve; *b.m.n.*, branchial main nerve; *b.n.f.*, branchial nerve-fibres to osphradium; *b.v.p.*, blood-vessel of principal filament; *c.*, cilia; *c.a.*, ctenidial axis; *ch.*, chitin; *cu.*, cuticle; *c.c.m.*, criss-cross muscle-fibres; *c.d.*, ciliated discs; *c.t.*, connective tissue; *c.p.g.*, cerebro-pleural ganglia; *c.p.c.*, cerebro-pleural commissure; *c.p.co.*, cerebro-pedal connective; *e.l.p.*, exterior labial palp; *e.*, endothelium; *f.c.*, frontal cilia; *f.n.*, filament nerve; *i.l.j.*, inter-lamellar junctions; *i.l.p.*, interior labial palp; *i.f.s.*, intra-filamentar septum; *l.c.*, lateral cilia; *l.c.m.*, lateral ctenidial muscle; *l.l.*, lower lip; *l.n.f.*, longitudinal nerve-fibres; *m.*, mouth; *m.c.*, mucus-cell; *m.f.*, muscle-fibres; *n.*, nerve-cells; *n.f.*, nerve-fibres; *n.p.*, nerve to palps; *o.*, osphradium; *o.f.*, ordinary filament; *p.f.*, principal filament; *p.g.*, pedal ganglia; *p.n.*, pedal nerve; *r.e.p.*, respiratory expansion of principal filament; *r.g.*, right gill; *s.*, spur; *s.c.*, sensory cell; *s.n.*, subsidiary nerve; *s.n.f.*, subsidiary nerve-fibres; *t.m.f.*, transverse muscle-fibres; *u.l.*, upper lip.

PLATE 16.

Fig. 1.—Nervous system, as seen from the left side, showing the nerve-supply of the mouth, palps and gills.

Fig. 2.—A portion of the principal filament with two ordinary adjacent filaments on either side, showing the layer of nerve-cells, stained intravitalum with methylene-blue.

Fig. 3.—A portion of the stratum of nerve-cells from gill-axis, showing felt of nerve-fibres and also the axones.

Fig. 4.—Transverse section of an ordinary filament.

PLATE 17.

Fig. 5.—Transverse section of filament near the free margin of the gill, showing both demibranchs with inter-lamellar junctions; the ordinary filaments adjacent to the principal filaments bear spurs.

Fig. 6.—Photomicrograph of part of a transverse section of principal filament and spurred adjacent filaments.

Fig. 7.—Transverse section of principal filament near base, showing respiratory expansions and the nerve-supply.

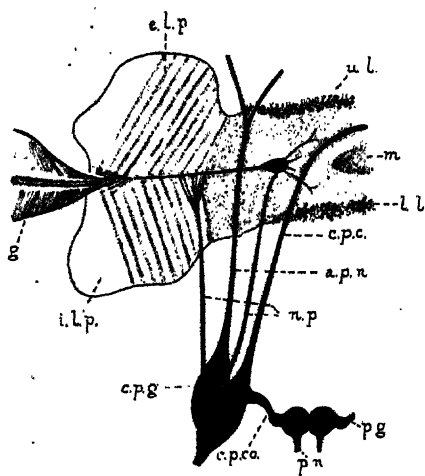
Fig. 8.—Transverse section through base of gill showing arrangement of muscle-fibres and the position of the subsidiary branchial nerve.

PLATE 18.

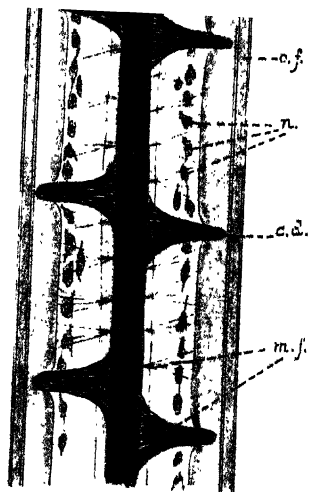
Fig. 9.—Transverse section of osphradium as seen on gill-axis.

Fig. 10.—Similar section.

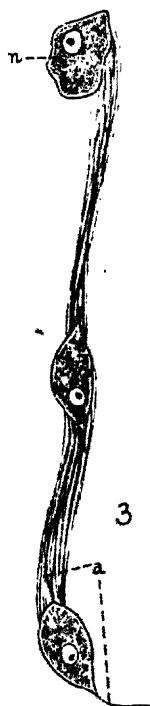
Fig. 11.—Section of the gill-axis. The figure shows two regions. Region A has been cut transversely and shows the fibres from the main branchial nerve. Region B is longitudinal horizontal; a large bundle of nerve-fibres cut longitudinally is seen below the cuticle of the epidermis; one bipolar cell is present.



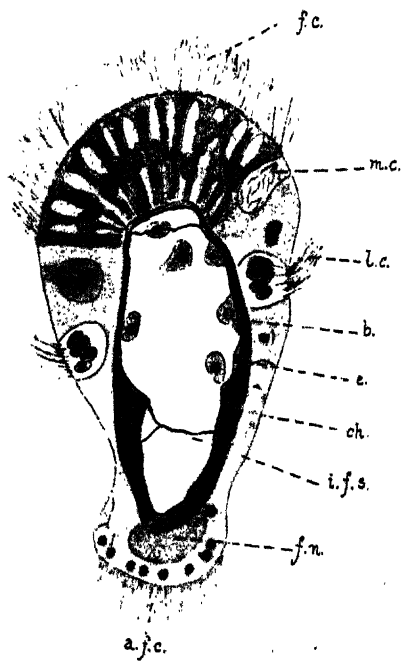
1



2



3



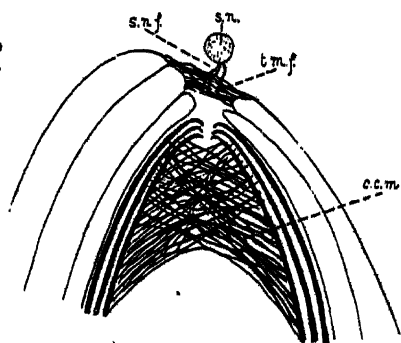
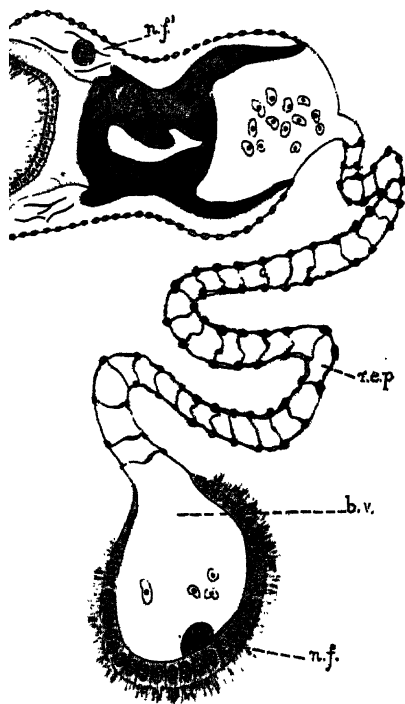
4



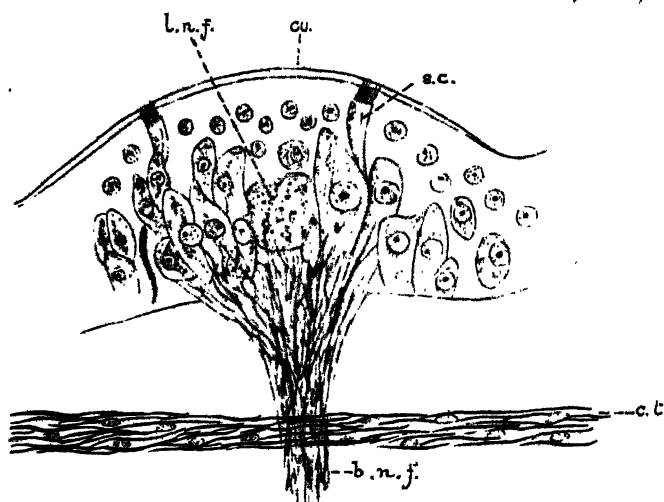
5



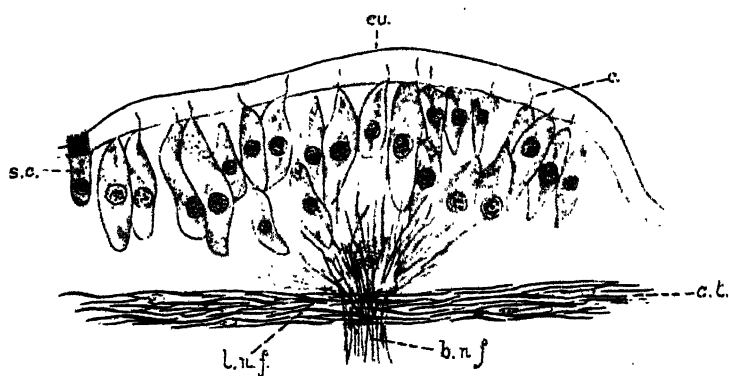
6



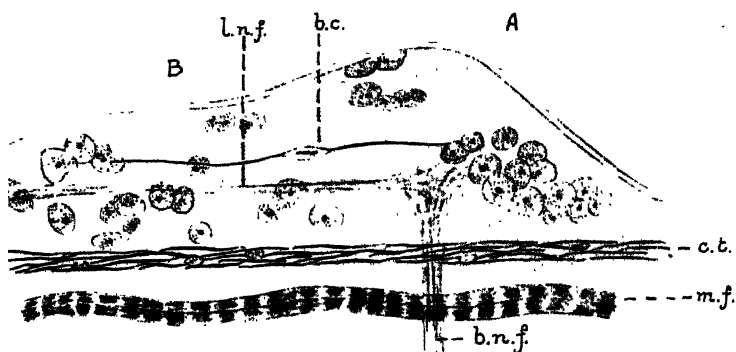
8



9



10



Metamerism in Enteropneusta.

By

Dr. C. J. van der Horst,

University of the Witwatersrand, Johannesburg.

With 3 Text-figures.

THE Chordate affinities of the Enteropneusta were advocated first by Bateson, who even included this group of animals amongst the Chordata. Most subsequent investigators of the Enteropneusta (MacBride, Willey, Ritter, and Heider in particular) supported Bateson's conception and even tried to give it more evidence. On the other hand, Spengel, the accepted imperator in the knowledge of the group, as Ritter rightly observes, denies all relationship between the Enteropneusta and the Chordata; he believes that *Balanoglossus* is distantly allied to the Annelida, and that the *Tornaria* is a modified Trochosphere. If the relations of the Enteropneusta with the Pterobranchia, Phoronis, and Echinoderms be left out of the question, as these can be considered to be mere side-lines in the course of evolution, it becomes apparent that the Enteropneusta, either through their relation to the Annelida or to the Chordata, are allied to segmented animals.

Spengel found no support for his view that the Enteropneusta are related to the Annelida, and MacBride in his review of Spengel's monograph has given good evidence that Spengel's conception rested on no solid foundation and is therefore of no value. The Chordate affinities of the Enteropneusta, on the other hand, are well established and generally accepted nowadays. In his last publication on the Enteropneusta in 'Handwörterbuch der Naturwissenschaften', Spengel himself seems to be inclined to leave at least room for the relationship between Enteropneusta and Chordata.

If the Enteropneusta are related to segmented animals, as

the Chordata are, the question arises whether there is any evidence of metamerism in *Balanoglossus*. The body consists of three parts: proboscis, collar, and trunk, each with its own coelomic cavity; the same arrangement is found in *Pterobranchia*, *Sagitta*, the *Actinotrocha*, and *Echinoderm* larvae. An indication of this trimerism is found, according to van Wijhe, also in the early development of *Amphioxus*. In *Balanoglossus* this metamerism is to some extent obscured by the great length of the trunk. If the posterior region of the animal had the same length as the collar, as Morgan suggests—and in early post-larval life they are equal in length—no one would doubt that we were dealing with a metameric animal consisting of three segments. The question naturally arises whether the long trunk of *Balanoglossus* really represents a single segment. If we accept that segmentation is largely due to the mode of locomotion, it becomes clear why a further segmentation of the trunk has either not been developed or has been lost in the *Enteropneusta*. Here locomotion is effected for the greater part by the action of the proboscis and the collar. According to Ritter the whole body is drawn forward, especially in *Dolichoglossus* with its long proboscis, by means of the contraction of the longitudinal muscles of the proboscis and the collar. Thus, if traces of metamerism are to be found in the trunk, one should not expect to find them in the coelom nor in the muscles, though these structures are most important in the segmentation of the *Annelida* and the *Chordata*. Thus real segmentation is excluded in *Enteropneusta* and only pseudometamerism in some form or other can be expected to be seen.

The gills, numbering usually from 50 to 100 pairs, form a regular row at both sides in the anterior part of the trunk. These show a metameric arrangement, but this alone is not sufficient proof for metamerism. If this branchiomeres has anything to do with metamerism in the trunk, other organs, coinciding in their arrangement with the gills, will have to be looked for. Of course, these organs must be in themselves independent of the gills. This excludes the vascular system at once. The branchial veins and arteries, of course, show the same

arrangement as the gills, and when the cutaneous vessels open into the branchial veins, as is the case for example in *Glandiceps talaboti*, their more or less metameric arrangement is also of no further interest. The nervous system shows no trace of metamerism at all, as could hardly be expected. Thus there are left only the intestine, the skin, and the gonads.

There is, connected with the intestine, besides the gills, another structure that shows a metameric arrangement to some degree. A number of sacculations, protruding at the dorso-lateral sides of the trunk, are present in a certain region of the gut. These are noticed in the Ptychoderidae, but are absent in the Harri-manidae, and in the Spengelidae they may be present or not. These hepatic caeca are placed in two regular rows and give the impression of a metameric arrangement. As the liver region is always situated far behind the branchial region, the arrangement of the sacculi cannot be compared with that of the gills, and taken alone these caeca give as little proof of metamerism as the gills.

The epidermis shows a certain zonulation, having the glandular cells arranged in zones separated by interannular depressions with or without a small number of glandular cells. Kowalewsky found that the number of these epidermal rings practically corresponds to the number of gills in *Glossobalanus minutus*, but in *Balanoglossus clavigerus* he found far more rings than gills. These rings are not limited to the branchial region; they extend over the whole length of the trunk, and in the abdomen they may be even more pronounced than in the branchial region. Willey expressed the opinion that this epidermal zonulation has not been well considered, and has been unjustly treated as having no deep-lying significance at all. His view, that the gill-slits originally arose as perforations in the interannular grooves, was opposed by Spengel, though a segmentation of the epidermis would fit in very well in his supposed relationship between Enteropneusta and Annelida. Spengel's objection, that the zonulation is produced only by a certain arrangement of glandular cells, is of no value, as glandular cells may show a metameric arrangement as well as

any other organ, and in some animals they actually show this. Spengel has pointed out that the zonulations, even in such an animal as *Ptychodera flava*, where they seem to have a fairly regular arrangement, show too many irregularities at closer examination, in order to give sufficient proof of a metamerism. It is probably better to leave these epidermal zonulations out of the question until an *Enteropneust* is found in which they are really metamerically arranged, than to build up theories around supposed structures.

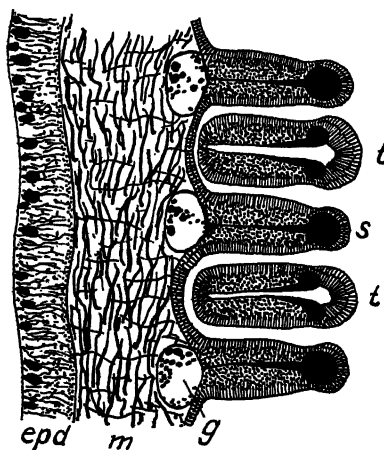
Lastly come the gonads. Gills and gonads are relatively independent structures, and the genital and branchial regions coincide to a large extent in the greater number of *Enteropneusta*. This makes the arrangement of the gonads a matter of great importance for the present question. Spengel could not find any relation at all between the arrangement of the gonads and the gills in the very extensive material that he studied, and thus expresses the opinion that the gonads cannot be looked upon as segmental organs, and, if they are, that the gonadomeres nevertheless do not correspond in any way to the branchiomeres. It is somewhat surprising that MacBride in his review of Spengel's monograph figures, apparently without any other excuse than the possession of a foreseeing mind, a schematic horizontal section of *Balanoglossus* in which the gonads regularly alternate with the branchial sacs. Willey, also, without giving any further evidence, accepted a regular alternation of gonads and gills, the gonads having a zonary disposition and the gill-slits occupying the interzonal depressions. On this supposition he based his theory that the primary function of the gill-slits is the oxygenation of the gonads, and their secondary function being the respiration of the individual. This is one of Willey's rather wild theories of which Spengel says: 'Es wird einem ja schwindlich, wenn man an diesen babylonischen Turmbau nur denkt.'

Yet there is a relation between the arrangement of the gill-slits and the gonads. Branchiommerism and gonadomerism coincide. Meek found this realized in *Glossobalanus marginatus*, where a certain region bears the same number of gonads as

gills. The same holds true for young specimens of *Glossobalanus crozieri*; gonads and gills are present in the same number, and to each branchial pore a genital opening is present, of course, only as far as the genital and branchial regions overlap.

The most indubitable evidence is given by *Dolichoglossus caraibicus*. This species has over fifty pairs of gill-slits. The first gonads are found between the fourth and fifth gill-slits at both sides of the body, and then a regular alternation

TEXT-FIG. 1.



Dolichoglossus caraibicus. Part of an horizontal section of the branchial region. $\times 66$.

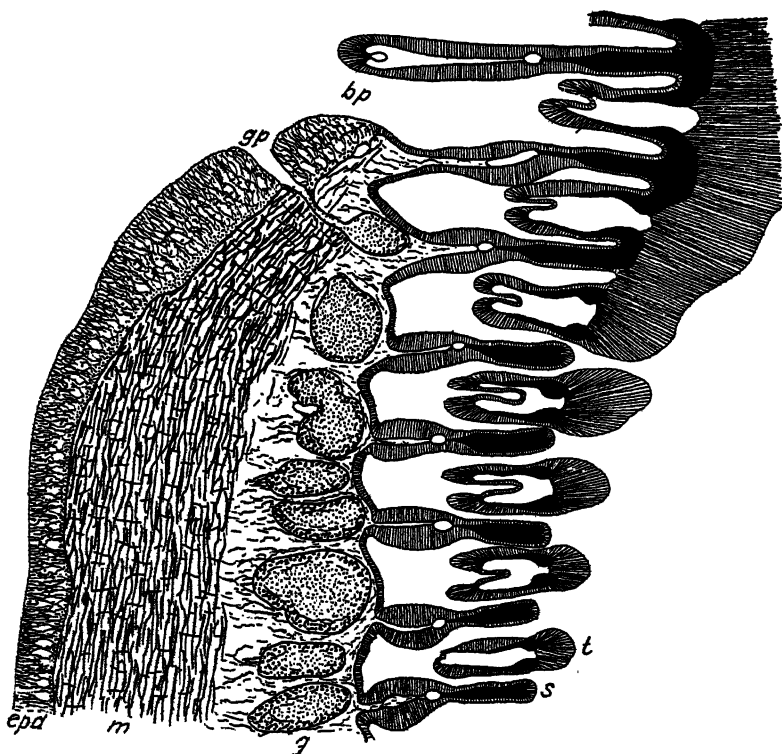
bp, branchial pore; *epd*, epidermis; *g*, gonad; *gp*, genital pore; *m*, musculature in body cavity; *s*, branchial septum; *t*, branchial tongue.

of gonads and gills is found till the end of the branchial region, so that at the peripheral side of each septum a gonad is situated (Text-fig. 1). The genital pores are found exactly between the succeeding branchial apertures. This relation between these two organs is very clear in *Dolichoglossus caraibicus*, as the gonads are very small and simple in the only available specimen.

The same phenomenon is found in *Glandiceps talaboti*, though a closer examination is necessary here, because the gonads

are large and branched. Near the external orifice the gonad is situated at the outer side of a gill-septum, but soon it becomes broader, extending to the back of the branchial sac (Text-fig. 2).

TEXT-FIG. 2.

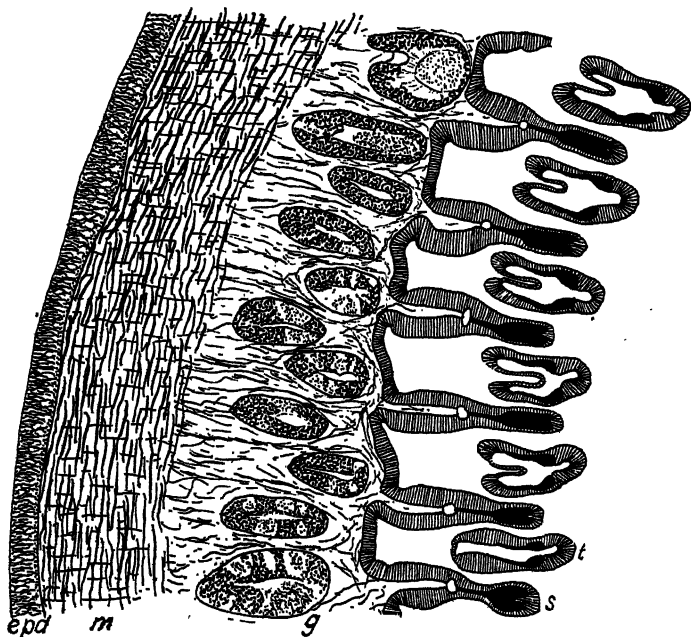


Glandiceps talaboti. Part of an almost horizontal section of the branchial region at the level of the pores. $\times 40$.

A little more ventrally the gonad branches into two, and these branches occupy a very definite position with regard to the gills; one is situated outside a septum, the other at the back of the branchial sac (Text-fig. 3). In the ventral part of the body, where the gonads may branch repeatedly, this relation between gills and gonads is quite disturbed.

Thus there are four species, representatives of the three families of Enteropneusta, in which the gonads alternate with the gills, that is in which gonadomerism corresponds to branchiommerism. Though no trace of this relation has been found in the

TEXT-FIG. 3.



Glandiceps talaboti. Part of an almost horizontal section of the branchial region, ventral to that figured in Text-fig. 2. $\times 40$.

majority of Enteropneusta, it does not appear to be too hazardous to accept this relation as an original character of the Enteropneusta, a character that has, however, been lost in some way or other in most of them.

There is a species that gives an indication of one way in which this arrangement has been lost. This species is *Dolichoglossus otagoensis*. The small number of gill-slits, about ten, does not make it very suitable for the purpose, nevertheless it

shows that there is some relation between the gills and the gonads. The small gonopores are in the same, or nearly the same, section in which the front end of the branchial pore is found. As the branchial pores are very wide and the space between them is correspondingly narrow, it follows that the gonopores are situated at the same level as the septa. Thus to every septum, except the first two, there is a corresponding gonad. In a young specimen with only six gill-slits this was found without exception. In older specimens more gonads may be present; thus in a specimen with ten gill-slits there were found two extra gonads, not situated at the level of a septum, in addition to those at the sides of the third to the tenth septa (the caudal wall of the last slit being considered as a septum). These gonads, however, were very small and apparently secondarily formed. Spengel described a new formation of gonads intercalated between the older ones in *Harrimania kupfferi*, and the same is found here in *Dolichoglossus otagoensis*. When these gonads mature, they push away the primary ones, in this way disturbing the original relation between gonads and gills.

Another manner in which the original relation may have been lost is that the gills lost their metameric arrangement in the same way as this occurs in *Amphioxus*. On comparing, e.g., the genera *Balanoglossus* or *Ptychodera* with *Dolichoglossus* or *Harrimania*, it is striking to notice that in the former the gills are far more crowded. As it is impossible to give any proof of this supposed compression of the gills by comparison with other organs, this is only a supposition.

In Vertebrates branchiomerism does not correspond to the general segmentation of the body, but in *Amphioxus* it does. Furthermore, *Amphioxus* shows, at least during the earlier stages of its development, exactly the same relation between gills and gonads as mentioned above for some Enteropneusta. Also in the young *Amphioxus* the gonads are situated between the gill-slits, i.e. opposite the branchial septa. This may be considered to be another proof of Chordate relationship of *Balanoglossus*, as this was accepted first by Bateson.

As the coelomic cavity of Enteropneusta, besides the trimerism in prostomium, collar, and trunk, does not show any further tendency to segmentation, this arrangement of the gills and gonads can be considered only to be a case of pseudometamerism. Compared with their distant allies, the Chordata, the Enteropneusta are, undoubtedly, very primitive animals; even *Amphioxus* shows a far higher organization. If the Enteropneusta are related to completely segmented animals, as the Chordata, it is possible that their pseudometamerism, which can be considered to be an original character of the group that has been lost in the majority of them, is only a relic of a former stage in which segmentation was more complete. It might have been lost in relation to their mode of life and locomotion in the same way as this can be accepted for Echiuroids. Another possibility, put forward by Heider, is that the metamerism of the vertebrate-body has as its distant predecessor the pseudometamerism of the Enteropneusta, and taking into consideration that the other relations of the Enteropneusta do not prove anything beyond the original trimerism, I am inclined to share this opinion of Heider.

LITERATURE.

- Bateson, W.—“On the morphology of the Enteropneusta”, ‘Quart. Journ. Micr. Sci.’, vol. 25, 1885.
- Heider, K.—“Phylogenie der Wirbellosen”, ‘Die Kultur der Gegenwart’, 3. Teil, 4. Abteilung, 4. Band. Leipzig, 1914.
- Kowalewsky, A.—“Anatomie des Balanoglossus Delle Chiaje”, ‘Mém. Ac. Imp. Sc. St-Petersbourg’, Sér. 7, tom. 10, 1866.
- MacBride, E. W.—“A review of Professor Spengel’s monograph on *Balanoglossus*”, ‘Quart. Journ. Micr. Sci.’, vol. 36, 1894.
- Meek, A.—“*Glossobalanus marginatus*, a new species of Enteropneusta from the North Sea”, *ibid.*, vol. 66, 1922.
- Morgan, T. H.—“The development of *Balanoglossus*”, ‘Journ. Morph.’, vol. 9, 1894.
- Ritter, W. E.—“Papers from the Harriman Alaska Expedition. II. *Harrimania maculosa*, a new genus and species of Enteropneusta from Alaska, with special regard to the character of its notochord”, ‘Proc. Washington Ac. Sc.’, vol. 2, 1900.

- Ritter, W. E.—“The movements of the Enteropneusta and the mechanism by which they are accomplished”, ‘Biol. Bull.’, vol. 3, 1902.
- Spengel, J. W.—“Die Enteropneusten des Golfes von Neapel. Fauna und Flora des Golfes von Neapel”, ‘Monogr.’, 18, 1893.
- “Neue Beiträge zur Kenntniss der Enteropneusten. I. *Ptychodera flava* Eschsch. von Laysan”, ‘Zool. Jahrb. Abt. Anat.’, Bd. 18, 1903.
- Willey, A.—“Enteropneusta from the South Pacific, with notes on the West Indian species”, Willey’s ‘Zool. Results’, Part 3. Cambridge, 1899.
- Wijhe, J. W. van.—“Studien über *Amphioxus*. I. Mund und Darmkanal während der Metamorphose”, ‘Verh. Kon. Ak. Wetensch. Amsterdam’, Deel 18, 1914.

The Cytology and Binary Fission of *Peranema*.

By

Virginus E. Brown,

Zoological Laboratory, University of California.

With Plates 19-21 and 1 Text-figure.

CONTENTS.	PAGE
INTRODUCTION	403
MATERIAL AND TECHNIQUE	404
MORPHOLOGY	405
MITOSIS	410
Prophase	410
Metaphase	411
Anaphase	411
Telophase	412
DISCUSSION	412
Staborgan	412
Kinetic Elements	413
Cytoplasmic Inclusions	415
GENERAL SUMMARY	416
REFERENCES	417
EXPLANATION OF PLATES	418

INTRODUCTION.

THE *Peranemidae* are animals of a primitive type and they show rather close phylogenetic relationships to the lower protistan forms. *Peranema* affords good cytological material. They have a clear cytoplasm and therefore their kinetic elements are easily determined. Likewise they are easy to culture. Thereby they can be obtained in quantities large enough to allow of good cytological investigation. Also the study of their cytology is of interest, for the conception of the Protozoa as a primitive type has led to various studies which may bring to light a better interpretation of the riddles of protozoan morphology.

The divergence of opinion among those who have written on the various flagellates belonging to the Euglenoidina has presented many interesting problems. The kinetic elements, the gullet, and the reservoir system of *Peranema* are subjects of controversy. The purpose of this paper is to clear up the various interpretations that have been made on the morphology and mitosis of this protozoon. It is also my aim to point out the phylogenetic relationships of the neuromotor system.

This work was done in the laboratory of Dr. Robert C. Rhodes at Emory University. I wish to express to Dr. Rhodes many thanks for his valuable criticisms and general co-operation. Thanks are due also to the University of California for supplying the optical equipment used in completing this investigation.

MATERIAL AND TECHNIQUE.

I have successfully cultured *Peranema trichophorum* in the following manner: A 200 c.c. culture of *Euglena proxima* was centrifuged and the material thus obtained was washed in distilled water and crushed between two glass slides. To this crushed *Euglena* I added 100 c.c. of tap-water and allowed the culture to stand for a day before I inoculated with *Peranema trichophorum*. After eight days division forms were observed in abundance.

For fixation, the centrifuge method of killing was found to be the best process as it ensured large quantities of the material. Various fixing reagents were used, but the best for general purposes were found to be hot Schaudinn's and strong Flemming's fixatives. I used the fixatives of Champy and Mann-Kopsch for demonstrating mitochondria. The most satisfactory nuclear stain was found to be Heidenhein's iron-alum-hematoxylin. Counter stains of eosin or Bordeaux red were used to demonstrate the axial filaments of the flagellum. 'Licht Grün' was used to show the spiral striations of the cuticle. The mitochondria were stained with fuchsin and toluidin blue after Champy's fixative, or they were impregnated with Bowen's modification of the Mann-Kopsch procedure. Then I stained with safranin to bring

out the nuclear structures. The method of Bowen likewise brought out the Golgi apparatus.

The plates were sketched by the aid of a camera lucida from a Busch and Lomb binocular microscope with 2 mm. apochromatic objective and $\times 12.5$ oculars.

MORPHOLOGY.

The genus *Peranema* is characterized by the lack of an eyespot. It is spindle or cigar-shaped, and it tapers anteriorly to a point. Its broad posterior end is truncate or retuse.

The size of *Peranema trichophorum* is $60\ \mu$ to $72\ \mu$ in length and $28\ \mu$ to $32\ \mu$ in breadth. It has a thin flexible cuticle of a metabolic nature. A single flagellum occurs, and it possesses two axial filaments which arise from basal granules situated at the base of the reservoir. The length of the flagellum varies from one-fourth to almost body length.

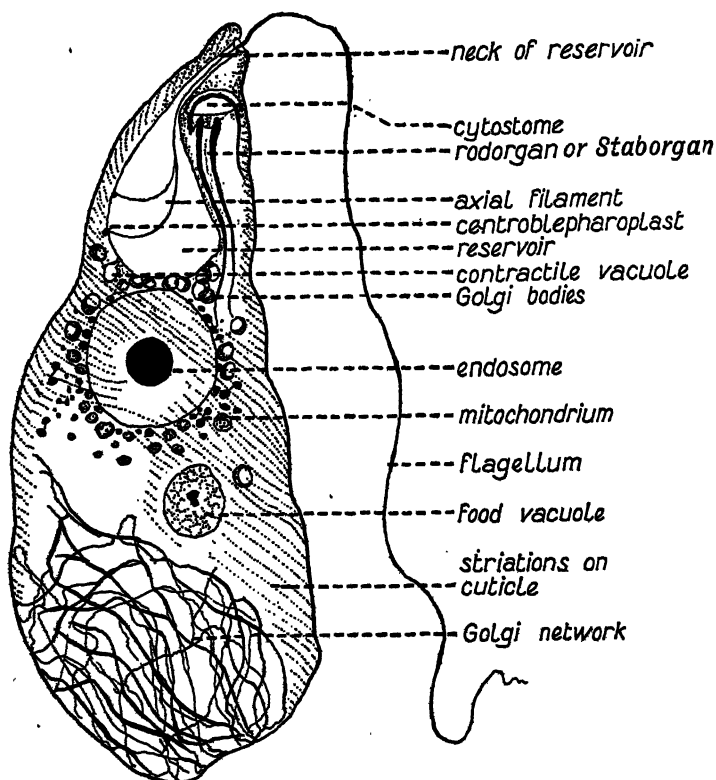
The genus is likewise characterized by a 'crawling' method of locomotion. Here the contour of the animal assumes various protean forms by means of a series of wave-like contractions and contortions of a fugacious character which run along the cuticular surface. These are clearly apparent when the animal doubles on itself by an abrupt retrogressive motion, which is similar to that described by Mast, 1922. The flagellum is usually kept straight forward and rigid, except the tip, which is bent into the form of a hook. This bent portion vibrates in a continuous whirl. The point of the flagellum when in motion describes an ellipse. *Peranema granulosa* often swims by whirling the entire flagellum around and hurls itself backward.

The vacuole system is complex; it consists of a large flask-shaped vesicle (= Hauptvacuole of Klebs, 1868); also a small contractile vacuole is situated at the base of this structure, which I prefer to call the reservoir after Rhodes, 1926; Baker, 1926; Calkins, 1926. At each systole the contents of this adjacent contractile vacuole (= Neben vacuole of Klebs, 1868) is emptied into the main reservoir. A slight contraction of this main reservoir seems to aid the flagellate to reach the substratum; so we suggest that its function is not only an organelle for the

storage of waste materials, &c., but also that it is of a hydrostatic nature.

I believe that the old family Peranemidae (Stein) should stand, because all of the group, with the exception of *Euglenopsis*,

TEXT-FIG. 1.



A diagrammatic sketch of *Peranema trichophorum*.

(Note that the crescent-shaped Golgi bodies shown around the reservoir are not present when the Golgi network occurs.)

possess a rod-organ, or 'Staborgan'. Hall and Powell were unfortunately not informed of this fact. The rod-organ of *Pelatomonas* is small and the lateral rods are quite short. Also

Scytomonas, *Tropidoscyphus*, *Marsupiogaster*, and *Dinema* have well-developed rod-organs. In *Urceolus* the rod-organ is spread out into an urn-like structure; whereas the rod-organs of *Anisonema* and *Entosiphon* extend posteriorad, as an internal 'siphon', almost the entire length of the body. I see no reason, therefore, to split up an old family of Peranemidae into several families which have one or more flagella. Shaeffer (1916) stated that the Peranemidae are holozoic and ingest solid organic matter which is composed of pieces of plant or animal tissues. I wish to add that they exhibit great selectivity with relation to their food.

Peranema trichophorum lives almost entirely on dead *Euglena proxima*, and has been observed to engulf *Entosiphon* and *Chilomonas*. The animal rarely if ever attacks immotile forms, but it is content to tear encysted or dead *Euglena* to bits. On the other hand, *Peranema granulifera* eats living *Zoochlorella*. This type of food selection is not uncommon among protozoa; for example, *Didinium* feeds almost entirely upon *Paramecium* (Calkins, 1926). It is quite reasonable to believe that *Peranema* is almost entirely holozoic, because a culture will not live unless it is inoculated with some Euglenoid. For this reason as well as the one mentioned above, I do not think it advisable to place *Peranema* in the same family with the saprozoic *Astasidae* of Calkins (1926), but I prefer to accept the classification of Doflein and place the genus in the old family Peranemidae (Stein).

Wager (1900) was the first to describe the insertion of a Euglenoid flagellum. He stated that the flagellum of *Euglena viridis* bifurcated on entering the main vacuole (= reservoir), and that each of these strands was anchored at the base of this vesicle to a basal granule. Later writers (Rhodes, 1926; Baker, 1926; Ratcliffe, 1928) have suggested that these strands be called axial filaments.

Hartman and Chagas (1909) believed that the flagellum of *Peranema* was single and that it was anchored at the base of the main vacuole (= reservoir) by a basal granule.

They also stated that another short flagellum arose within

this vesicle from a basal granule adjacent to the other one and traversed the vesicle to become anchored to another granule on its upper surface.

Hall and Powell (1928) believed that the flagellum remained single after entering the reservoir and that a new flagellum started to grow out of the reservoir early in mitosis.

Doflein figures *Peranema* with a single flagellum which has two axial filaments similar to those described by Wager (1900) for *Euglena viridis*.

It is possible that Hartman and Chagas (1909), as well as Hall and Powell (1928), misinterpreted the second axial filament as a new flagellum of the daughter individual. I find that *Peranema* has a flagellum which is formed by the union of two axial filaments which arise from basal granules situated at the side of the reservoir.

Peranema does not possess the granule which has been described to occur on one of the axial filaments by Wager, 1900; Baker, 1926; and Ratcliffe, 1927; however, in all other respects its flagellum is similar to that of *Euglena*. It is possible that this granule is absent in *Peranema* because it does not possess an eye-spot. Wager (1900) believed this granule was associated with the eye-spot of *Euglena* and that it aided in orienting the animal to light. Since this granule is absent in *Peranema* it is possible that this statement is correct.

The occurrence of a cytostome has been detected by Carter, Clapède, and James-Clark, but Klebs (1888) gave the first description of its organization. He believed that the mouth opening was ventrally placed and that it was separated from the 'Hauptvacuole' or reservoir. However, he failed to consider the pharynx as a tubular structure, and he believed it was composed of two adjacent rods on the ventral side of the cuticle. These he termed the 'Stabapparat'.

Rhodes (1926) has shown that the reservoir of *Heteronema acus* is a separate structure from the 'staborgan' (Stabapparat of Klebs), and that it consists of three rods. The outer lip of the cytostome is bounded by a falcate rod, which he terms 'trichite'.

He stated that the tube which leads from the mouth is bounded on each side by lateral rods.

I agree with Klebs (1888) and Rhodes (1926) that the 'staborgan' and the gullet of *Peranema* are not connected in any way with the reservoir, but that the cytostome is a separate opening on the ventral side of the body. The cytostome is capable of great distension and its outer lip is reinforced with a heavy falcate rod. The inner or proximal 'lip' is protoplasmic. These rods of the 'staborgan' probably act as a support to strengthen the sides of the gullet and to prevent the prey from tearing its own cuticle. The third rod (= falcate rod or trichite of Rhodes, 1926) seems to act not only as a support to the lower lip, but also as a trigger or valve which prevents the cytostome from opening except when the protozoan is feeding. It also aids in holding its prey so that it can be pinched off by the aid of the other two rods which border the gullet (= Cytosophagus of Rhodes).

I agree with Rhodes, 1926, that the rod-organ is displaced during the early prophase and that it later disintegrates in the cytoplasm. New rod-organs are formed in the early anaphase. The new cytostomes are formed by an inpitting of the cuticle. At the base of this pit a granule is formed and out from it grow two rows of granules. These condense to form the lateral rods of the gullet. (I prefer to use this term because it has been used extensively in protozoology; however, it should not be confused with any part of the reservoir.) During the telophase the third or the falcate rod forms by an outgrowth from the top of the medial lateral rod (fig. 11, Pl. 20; fig. 12, Pl. 21).

The mitochondria of *Peranema* vary from a small round form to a large disc-shaped type with a clear centre. The small spherical forms range from $\frac{1}{4}\mu$ to $\frac{1}{2}\mu$, whereas the disc-shaped types vary from $\frac{1}{4}\mu$ to 1μ in width and are $\frac{1}{8}\mu$ to $\frac{1}{4}\mu$ in breadth. They are usually grouped around the nucleus and the base of the reservoir. When the protozoan divides most of the mitochondria move anteriorly along with the nucleus and group themselves about the reservoir. The ovoid mitochondria seem to be capable of becoming the disc-shaped types by growth.

These disc-shaped types show a differential staining with the toluidin blue and fuchsin method. The core is magenta, whereas the cortex is dark blue. The small mitochondria often show connexions or form dumb-bell structures. These are in all probability division stages. The mitochondria are straw-coloured when stained by the Mann-Kopsch method, whereas the Golgi apparatus is black.

The Golgi apparatus of *Peranema* is a network of long interwoven fibres which are concentrated in the posterior portion of the animal (fig. 15, Pl. 21). This network is not as dense in the later division stages of *Peranema* as it is during the early prophase. Neither the reservoir nor the contractile vacuole takes an osmic impregnation. However, the Golgi apparatus forms spheres around the reservoir during the early prophase.

MITOSIS.

Prophase.—During the early prophase the nucleus of *Peranema trichophorum* migrates anteriorly to the base of the reservoir. The nucleus at this time is of a vesicular nature; its centre contains a large dark staining body of chromatin. This body may be single or fragmented (Hall, 1926). This endosome (= Binnenkörper) is filled with vacuoles of various sizes. No centriole occurs within their centre, as some writers have believed. Therefore I agree with Hall and Powell (1928) that these vacuoles are of no significance. In the early prophase the dispersed chromatin comes together to form very thin chromomeres. These chromomeres are formed from 'spherules' which are connected together to form loops. Within these chromatic structures one can easily discern basophilic granules of variable sizes. During the growth of chromatin these loops thicken and the nucleus moves anteriorly and comes to lie on the edge or the base of the reservoir. By continued anterior migration it comes in contact with the largest basal granule (fig. 4, Pl. 19). This granule or centrophleharoplast seems to pull the nucleus upward and strands can be traced from it into the endosome. The endosome begins at once to elongate and the nucleus swings around on its axis until the other portion of

the elongated endosome is connected by similar lines to the other basal granule. The chromatin loops then thicken and arrange themselves parallel to the elongated endosome. At this period the longitudinal splitting of the metaphase occurs. The chromosomes separate from only one end to form V-shaped structures (fig. 5, Pl. 19).

At this stage a polarity is established between the opposite centropharoplasts at each end of the nucleus. Also fibres are noticed connecting each centropharoplast with the end of the elongated endosome (fig. 6, Pl. 19). The nucleus then pushes up against the reservoir. This not only distorts the vesicle but also it pushes the rod-organ out of position (figs. 5, 6, Pl. 19). The rod-organ is thus cast out into the plasma of the animal, where it disintegrates before the end of the anaphase (figs. 5, 6, Pl. 19, and fig. 7, Pl. 20). This disintegration of the rod-organ has been described in *Heteronema acus* by Rhodes, 1926.

During the prophase the basal granules divide twice to form new basal granules; out from these grow axial filaments. Each of these filaments unites with one of the old axial filaments to produce a new flagellum. A part of the flagellum thus persists to form one of the new flagellar filaments of a daughter individual. The chromosome count at this time has been estimated to be thirty-two.

Metaphase.—The longitudinal splitting of the chromosomes takes place during the prophase when the chromatin is arranged in a group of granular chromosomes. These pull apart to form V-shaped chromosomes (fig. 4, Pl. 19). These V-shaped structures widen and contract into chromosomes which come to lie parallel to the endosome (fig. 5, Pl. 19). Such a situation is usually termed the equatorial plate. The nuclear membrane remains intact.

Anaphase.—The endosome continues to elongate and often vacuolates or splits longitudinally (figs. 8, 9, 10, Pl. 20). The chromosomes during the early anaphase separate or pull apart from the last end which splits during the metaphase, and thereby an appearance of a pseudo-transverse splitting is noticed. The chromosomes become more granular and group themselves at

the ends of the endosome ; the nucleus then shows a constriction in the central portion (figs. 8, 9, 10, Pl. 20). The attachment between the endosome and the centropharoplasts persists.

During the anaphase the new cytostomes are formed by invagination of the anterior cuticular surface. On the edge of this newly formed cytostome a group of four granules grows in size and later they collect into two distinct rod-like structures (fig. 9, Pl. 20). These form the lateral rods of the rod-organ. The daughter reservoirs pull farther apart, and a partition begins to form between them. This division of the daughter reservoirs is produced by an outgrowth from the base of the old reservoir.

Telophase.—The telophase begins by a constriction of the anterior end of the animal. This deepens and the animal begins to split from the anterior end so that the animal divides by binary fission. The continued constriction of the nucleus causes the central portion to break apart. The chromosomes form granular loops around the endosome (fig. 12, Pl. 21). The third rod of the staborgan forms by an outgrowth from the top of the median rod. This connexion persists after the rod is formed; the same condition is found in *Heteronema* (Rhodes, 1926).

Division is completed by the daughter individuals pulling apart (fig. 13, Pl. 21). This action is very violent and the entire protoplasm is kept in motion. The flagella grow out, and they are kept in violent motion until the division is completed.

DISCUSSION.

Rod-organ or 'Staborgan'.—The Staborgan of *Peranema* consists of the rods which I have described above. There is no evidence that these rod-organelles are parabasals, as Calkins (1926) and Hall (1926) believed.

In fact *Peranema* seems to use them in feeding, and they are used just as a ciliate uses its trichites. The falcate rod opens and closes the cytostome when the animal feeds. Hall (1926) believed the parabasal to lie adjacent to the gullet and that the parabasal body doubled during division. Hall and Powell (1927) stated that they believed the parabasal body to be the equivalent of the 'Staborgan'. Later (1928) they ignore this statement

(Hall, 1926) and refer to it as the pharyngeal rod apparatus. I object to the use of this term since the cytostome is not connected with the reservoir, hence it cannot be of a pharyngeal nature. I suggest that the term 'Staborgan' or rod-organ (= rod organelle) be applied to this structure since this term is found in all of the literature (Doflein, 1912; Rhodes, 1926). Also I have not been able to find a parabasal in *Peranema*, and I see no reason to believe that the 'Staborgan' is either analogous or homologous to the parabasal body. Let us consider this question from a phylogenetic view-point. In the primitive protist, *Euglena*, we find a homology between the parabasal body and the kinetic complex (Baker, 1926). This kinetic complex is derived from the endosome. Now in a higher form like *Peranema* it is reasonable to believe that such a body is either lost during the evolution of this protozoon or that it still remains within the endosome. Obviously the endosome is part of the kinetic mass; I do not believe that *Peranema* has any homologue of the parabasal body, but I do believe that the endosome is the kinetic reserve mass.

Hall and Powell (1928) state that *Peranema* has only one 'pharyngeal' rod element in the early stages of binary fission. This they believe suggests that one of the two original rods passes to each of the daughter organisms. I have not noticed anything like this and I suggest that Hall and Powell studied the 'Staborgan' from a lateral view so that one would be above the other, and unless care is used this structure may appear as only one. The 'Staborgan' does not split during binary fission, but the old 'Staborgan' disintegrates and new ones form from granules on either side of the new daughter cytostomes. These granules are possibly of mitochondrial origin.

Kinetic Elements.—The blepharoplast-rhizoplast-centrosome complex is of a primitive type and has been described to occur in many of the lower flagellates. Likewise we find such a complex in most of the Polymastigotes and the Hypermastigotes. Associated with this centrophlepharoplast-rhizoplast-centrosome complex, in the Polymastigotes and the Hypermastigotes, is a dark staining paradesmose. This structure

connects the daughter centrosomes during fission (Kofoid and Swezy, 1915). I do not find such a paradesmose in *Peranema*. The kinetic elements of *Peranema* are of a type like that of *Ochromonas* (Doflein, 1912). Here we have the centrobalepharoplast, but no paradesmose occurs. During mitosis centrobalepharoplasts are connected to the ends of the endosome by numerous spindle-fibres. No permanent rhizoplast exists. In fact I see no reason to believe that the 'balepharoplast-rhizoplast-centrosome' complex occurs in *Peranema*; thousands of granules occur around the nucleus, but these do not function as a centrosome.

Although the paradesmose is known to occur in the *Hypermastigotes* and in the *Polymastigotes*, I suggest that it does not occur in any of the Euglenoid flagellates, because it has not been demonstrated by any of the recent workers on the Euglenoid group (Baker, 1926; Ratcliffe, 1928).

After a due consideration of the behaviour of the centrobalepharoplast of *Peranema* during mitosis, I have decided that there is a relationship between the centrobalepharoplast and the endosome. In other words, the action of the centrosome, or better, the centrobalepharoplast, does not initiate mitosis alone; but that this kinetic force is an interaction of both the centrobalepharoplast and the endosome as well as intranuclear physiological forces. If such a kinetic mass as the endosome is charged by a type of 'mitokinetism' or any type of electrostatic force, and if that force is associated with other forces which are of a physiological nature, there will be balance between all the forces which may initiate mitosis. Now if this balance is upset and a change in polarity occurs and the endosome is elongated, then this structure will split (fig. 8, Pl. 20). In all probability the same interacting forces cause the chromosomes to split longitudinally and to 'flow' apart. If the endosome is the centre of this kinetic force, then it is quite reasonable to expect that a division centre (or even a kinetic reserve complex) is given off in some of the lower protistan forms. Otherwise it would be hard to establish a phylogenetic line between *Peranema* and the lower flagellates. Such division centres or kinetic reserve

masses have been described by various workers (Kater, 1925 ; Baker, 1926).

The Cytoplasmic Inclusions.—Hall (1928) describes certain cytoplasmic inclusions which he believes are mitochondria and Golgi elements. The mitochondria he states are small elongated structures in *Peranema* and they lie in spiral rows. Likewise he noted numerous spherical inclusions similar in structure to the 'Golgi' elements of higher animals. I find that the mitochondria are rarely in spiral rows and that they assume this position for only a short while during the early prophase. It is possible that Hall (1928) has confused the Golgi network with the mitochondria, because at this time the Golgi apparatus is a network of long fibres. These fibres of the Golgi network are grouped in spirals round the nucleus and round the base of the reservoir (fig. 15, Pl. 21). When *Peranema* is not in division, the Golgi apparatus is a network of long fibres arranged in a tangled mass. During the early prophase these fibres condense into a tangled network which lies in the posterior portion of the animal. This mass breaks up into small irregular bodies which group themselves round the nucleus during the metaphase. But the typical network of long fibres form again during the anaphase and persist as such during the interkinetic phase. The mitochondria are spherical and disc-shaped (fig. 14, Pl. 21). The large disc-shaped types are grouped heavily round the nucleus and the base of the reservoir. They lie deep within the cytoplasm, whereas the spherical forms are more superficial and often take dumb-bell or rod-like shapes. The spherical forms are almost evenly distributed throughout the cytoplasm, whereas the large disc-types are grouped together. These disc-shaped types quite often show a clear centre. Rarely these disc-shaped types form 'roulettes'.

In the same slides with the *Peranema* material I found *Euglena proxima* and *Euglena gracilis*. The mitochondria are similar in shape and distribution to those I have described in *Peranema*. This leads me to believe that the mitochondria of *Euglena* described by Causey (1926) are only a part of the chromatophores and possibly the pyrenoids of this

protist ; however, I shall discuss the behaviour of the cytoplasmic inclusions of *Euglena* during binary fission in another paper.

GENERAL SUMMARY.

1. *Peranema trichophorum* is holozoic in nature and selective in its food, but not predaceous. It feeds usually on dead and encysted *Euglena proxima*, *Euglena gracilis*, and rarely upon *Chilomonas* and *Entosiphon*.

2. The 'Staborgan' or rod-organ is not connected with the reservoir, but it opens into the cytostome which lies ventrally to this vesicle. Therefore the term gullet should not be applied to the neck of the reservoir.

3. The chromosome count of *Peranema trichophorum* is estimated to be thirty-two in number.

4. The 'Staborgan' is thrown out of its position during mitosis and it disintegrates in the cytoplasm. New rod-organs grow out from granules which form at the base of the new daughter cytostomes. These granules may be of mitochondrial origin.

5. A centrolepharoplast is described. No paradesmose is present.

6. A theory is suggested which supposes that an interaction between the centrolepharoplast and the endosome occurs. The centrolepharoplast acts as a kinetic attraction sphere which carries the nucleus anteriorly in order that the blepharoplasts can function as extra-nuclear division centres ; thereby a co-ordinated interaction is brought about between both intra-nuclear kinetic elements and all of its cellular components. Such a reaction or interrelation of parts is necessary to initiate cellular division.

7. The mitochondria of *Peranema* were found to be spherical ; these may grow into large disc-shaped types with clear centres. The latter have a tendency to group themselves round the nucleus and the reservoir.

8. The Golgi apparatus was found to be a network of long fibres. These Golgi bodies seem to be concentrated in the posterior end and round the reservoir. Neither the contractile

vacuole nor the reservoir was impregnated by osmic acid methods.

REFERENCES.

- Alexeieff, A. (1911).—"Haplomitose chez les Eugleniens et dans d'autres groupes de Protozoaires", 'C. R. Soc. Biol., Paris', 71, 614.
- Baker, W. B. (1926).—"Studies in the Life-history of *Euglena*. I. *Euglena agilis*", 'Biol. Bull.', 51, 321-62.
- Bělař, K. (1916).—"Protozoenstudien. I. *Astasia levis*", 'Arch. Prot.', 36, 13-51.
- Blochmann, R. (1894).—"Über die Kernteilung bei *Euglena*", 'Biol. Centralbl.', 14, 81-91.
- Bowen, R. H. (1926).—"The Golgi Apparatus. Its structure and functional significance", 'Anat. Rec.', 32.
- (1928).—"The Methods for the Demonstration of the Golgi Apparatus. V. The Idiosomic Component, Methods for Lipoids, Trophospongium, Vacuome and Chromidia", 'Anat. Rec.', 40, 103-31.
- Bütschli, O. (1883-9).—"Protozoa." Bronn's 'Klassen und Ordnung des Thierreichs', 1, 1-2035.
- (1906).—"Beiträge zur Kenntnis des Paramylons", 'Archiv Prot.', 17, 197-226.
- Calkins, G. N. (1926).—"The Biology of the Protozoa", Lea and Febiger, 623.
- Causey, David (1926).—"Mitochondria in *Euglena gracilis*", 'Univ. of Calif. Publ. in Zool.', 28, 217-24.
- Dangeard, P. A. (1902).—"Recherches sur les Eugleniens", 'Le Botaniste', sér. 8, 1902, 96-357.
- Doflein, F. (1916).—"Lehrbuch der Protozoenkunde." Jena, G. Fisher.
- Hall, R. P. (1923).—"Morphology and Binary Fission of *Menoidium incurvum*", 'Univ. of Calif. Publ. in Zool.', 20, 447-76.
- (1926).—"Binary Fission in *Peranema trichophorum*", 'Anat. Rec.', 34, 155.
- (1928).—"Cytoplasmic Inclusions of *Peranema*", *ibid.*, 41, December.
- Hall, R. P., and Powell, W. N. (1927).—"A Note on the Morphology and Taxonomic Position of *Peranema trichophorum*", 'Tr. Amer. Micr. Soc.', 46, 155-65.
- (1928).—"Morphology and Binary Fission of *Peranema trichophorum*", 'Biol. Bull.', 54, 36-60.
- Hartmann, M., and Chagas, C. (1910).—"Flagellatenstudien", 'Mem. Inst. Osw. Cruz.', 2, 64-125.
- Hasse, G. (1910).—"Studien über *Euglena sanguinea*", 'Arch. f. Prot.', 20, 47-59.
- Kater, J. McA. (1925).—"Morphology and Life-history of *Polytomella citri*, sp. nov.", 'Biol. Bull.', 49, 213-237.

- Kent, W. S. (1880).—‘Manual of the Infusoria.’ London, Bogen, 1-472.
- Keuten, Jacob (1895).—“Die Kernteilung von *Euglena viridis*”, ‘Zeitschr. f. wiss. Zool.’, 60, 215-35.
- Kofoed, C. A. (1923).—“The Life-cycle of the Protozoa”, ‘Science’, 57, 397-408.
- Kofoed, C. A., and Swezy, O. (1915).—“Mitosis and Multiple Fission in *Trichomonad Flagellates*”, ‘Proc. Amer. Acad. Arts Sci.’, 2, 289-378.
- Lemmermann, E. (1913).—“Eugleninae”, in ‘Die Süßwasserflora Deutschlands, Österreichs und der Schweiz’, 2, 115-74. Jena, G. Fischer.
- Mast, S. O. (1912).—“The Reactions of the Flagellate *Peranema*”, ‘Journ. Anim. Behr.’, 2, 91-7.
- Minchin, E. A. (1912).—“An Introduction to the Study of the Protozoa”, xi, 520. London, Arnold.
- Mitchell, J. B., jr. (1928).—“Studies on the Life-history of a Parasite of the Euglenidae”, ‘Amer. Micro. Soc.’, xlvii, 1, 29-40.
- Nassonov, D. N. (1924).—“Der Exkretionsapparat der Protozoen als Homologe des Golgischen Apparates der Metazoenzellen”, ‘Arch. f. Mikrop. Anat. u. Entwickl.’, 103.
- Ratcliffe, H. L. (1927).—“Mitosis and Cell Division of *Euglena spirogyra*”, Ehrenberg. ‘Biol. Bull.’, 53, 109-18.
- Rhodes, R. C. (1926).—“Mouth and Feeding Habits of *Heteronema acus* (The mouth of the *Peranemidae* Klebs, involving the classification of the *Euglenoidina* relative to the new family *Heteronemidae* Calkins)”, ‘Anat. Rec.’, 34, 152-3.
- Schaeffer, A. A. (1918).—“A new diatom-eating Flagellate”, ‘Amer. Micr. Soc. Tr.’, 37, 177-82.
- Stein, F. P. von (1878).—‘Der Organismus der Infusionsthier’, Ab. III. Hälfte, 1, x-154. Leipzig, Engelmann.
- Steuer, A. (1904).—“Über eine *Euglenoide* aus Canale Grande von Triest”, ‘Arch. f. Prot.’, 3, 126-37.
- Tannreuther, G. W. (1923).—“Nutrition and Reproduction in *Euglena*”, ‘Arch. Entwickl. Mech.’, 52, 367-83.
- Tschenzoff, Boris (1916).—“Die Kernteilung bei *Euglena viridis*”, ‘Archiv f. Prot.’, 36, 137-73.
- Wilson, E. B. (1925).—‘The Cell in Development and Heredity’, xxxvi-1232. New York.

EXPLANATION OF PLATES 19-21.

Figs. 1-15 inclusive are of *Peranema trichophorum*.

All figures were drawn with the aid of an Abbe camera lucida. Figs. 2-4, 6-18, from preparations fixed in Schaudinn's

fluid and stained with iron-alum hematoxylin ; fig. 5 fixed in strong Flemming's and stained in safranin and light green ; fig. 1 fixed in strong Flemming's and stained with eosin. Fig. 14 fixed in Champy's and stained with fuchsin and toluidin blue ; fig. 15, Bowen's modification of the Mann-Kopsch procedure. Magn. $\times 1,800$.

PLATE 19.

Fig. 1.—Interphase. Ventral view showing cytostome opening bordered by falcate rod ; reservoir is below this and it opens at tip of body. Neck of reservoir not connected with cytostome.

Fig. 2.—Cytostome to left of reservoir ; endosome fragmented into four pieces ; nucleus migrates to base of reservoir.

Fig. 3.—Early prophase ; growth of chromatin ; blepharoplasts in normal position at side of reservoir. Posterior portion of reservoir vacuolated.

Fig. 4.—Left blepharoplast enlarges and becomes connected by strands to end of endosome ; it is therefore a centrobalepharoplast.

Fig. 5.—Metaphase. Chromosomes split longitudinally to form V-shaped structures ; endosome begins to elongate ; one large centrobalepharoplast is connected to end of endosome. 'Staborgan' is thrown out of place ; it disintegrates in cytoplasm below nucleus.

Fig. 6.—Chromosomes thicken ; nucleus swings on axis and connects with the other centrobalepharoplast ; new daughter reservoirs forming ; new axial filament on one side.

PLATE 20.

Fig. 7.—Anaphase. Chromosomes pulling to opposite poles. New rod-organs forming as granules around flagellar groove.

Fig. 8.—Anaphase. Endosome splits into two pieces ; new axial filaments unite to form new flagellum.

Fig. 9.—Top view. Lateral rods of rod-organs beginning to form ; endosome fragmented and split into two pieces.

Fig. 10.—Late anaphase, nucleus begins to constrict in central portion.

Fig. 11.—Late anaphase ; endosome not fragmented.

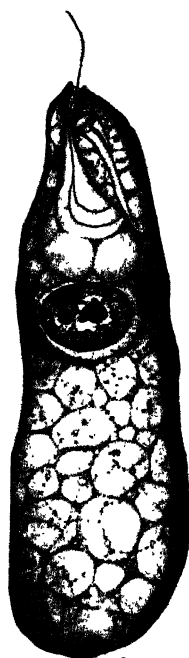
PLATE 21.

Fig. 12.—Telophase. Connexion between daughter nucleus is broken ; binary fission begins. Falcate rod forms.

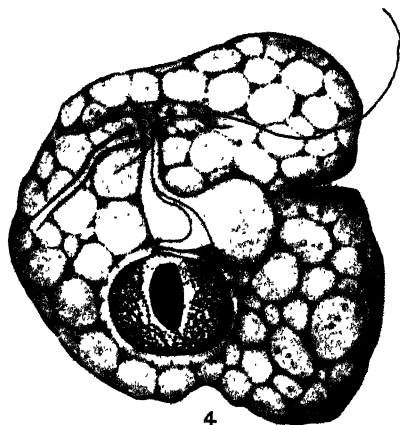
Fig. 13.—Telophase. Fission almost complete ; chromatin reorganized.

Fig. 14.—Mitochondria. Large disc-shaped types with clear centre grouped around reservoir and nucleus. Small dark dumb-bell types are possibly in division.

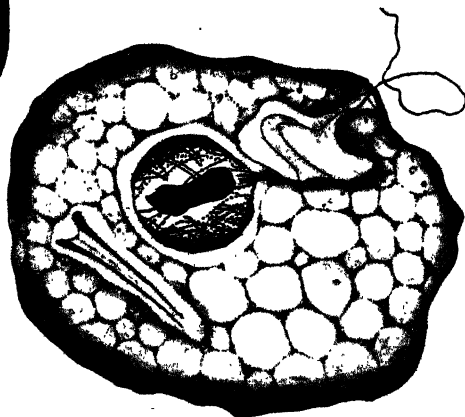
Fig. 15.—Golgi network of long fibres ; disc-shaped mitochondria distributed throughout cytoplasm.



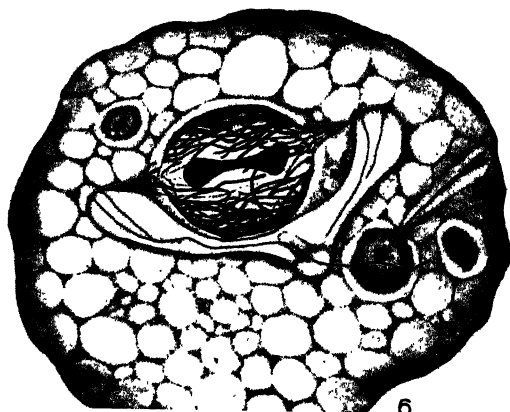
3



4

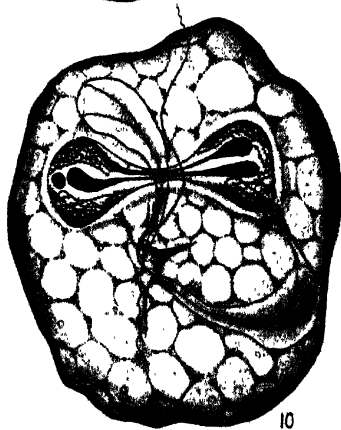
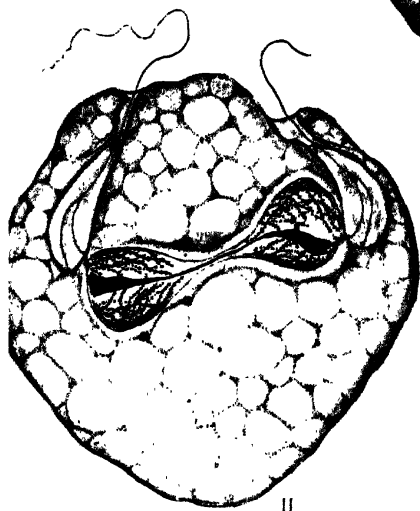
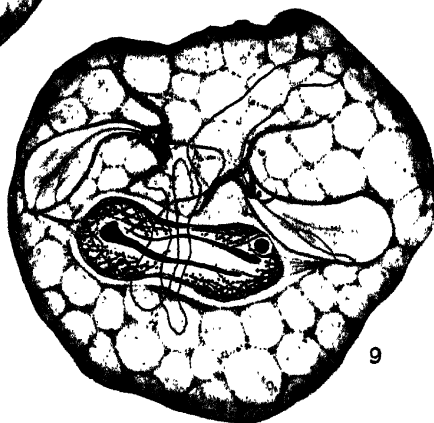
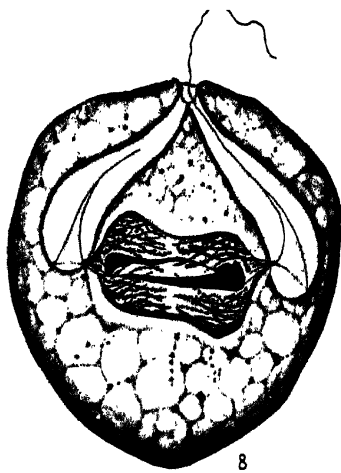
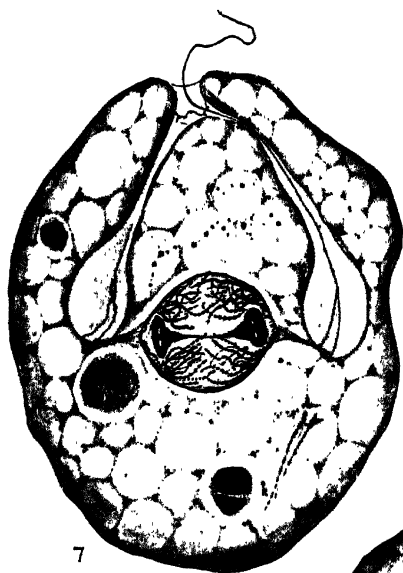


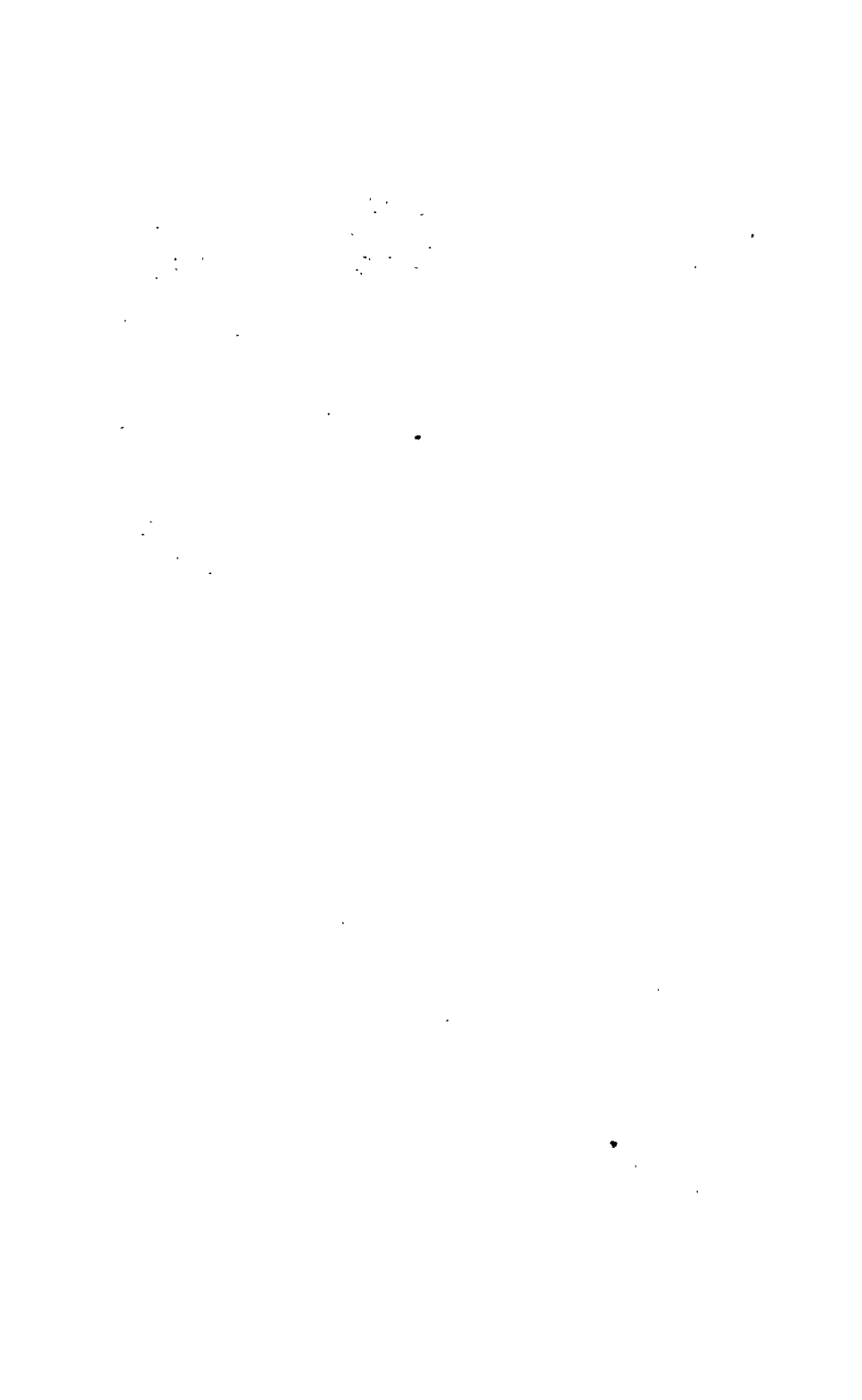
5

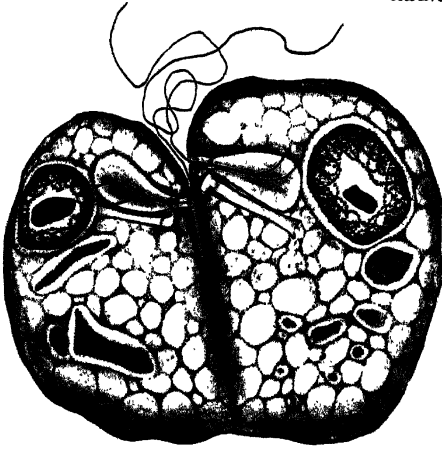


6

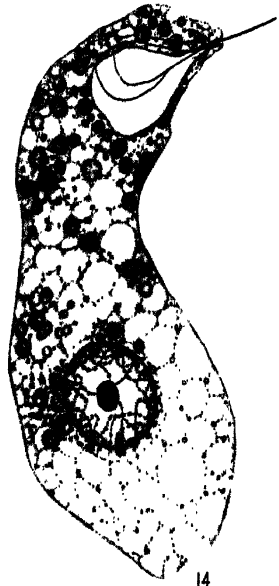




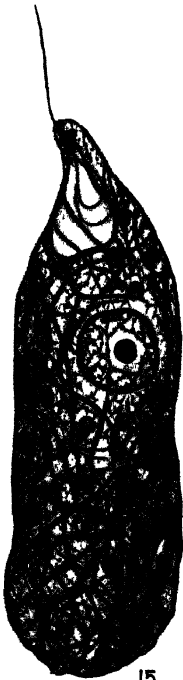




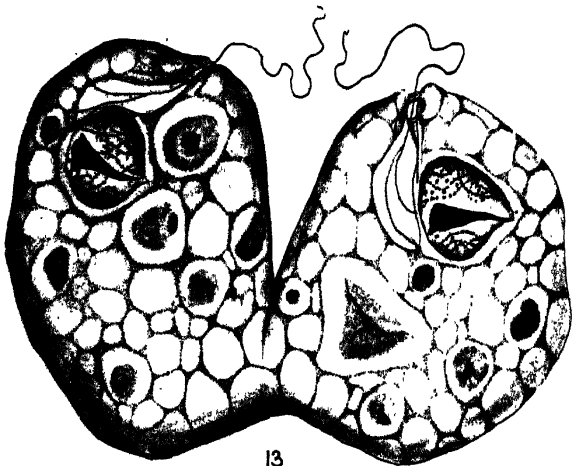
12



14



15



13

Preliminary Studies on the Bacterial Cell-mass (Accessory Cell-mass) of *Calandra oryzae* (Linn.): The Rice Weevil.

By

K. Mansour, B.Sc., D.I.C., Ph.D.,

Lecturer in Zoology in the Egyptian University, Abbassiah, Cairo.

With Plates 22, 23, and 4 Text-figures.

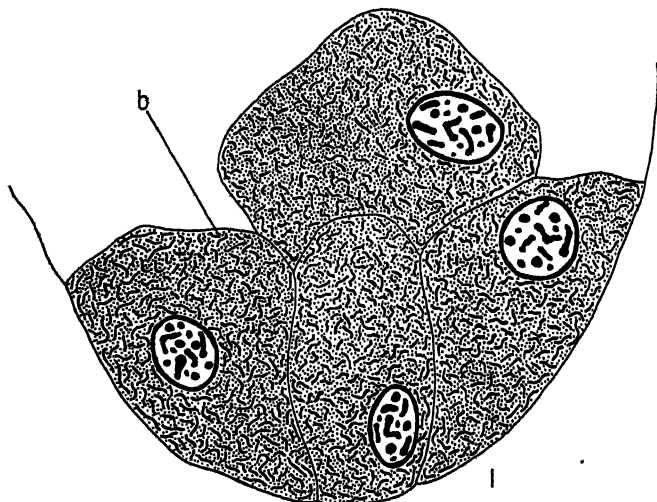
CONTENTS.

	PAGE
I. INTRODUCTION	422
II. TECHNIQUE	423
III. THE INTRACELLULAR BACTERIA	424
IV. THE 'ALIMENTARY BACTERIAL CELL-MASS' IN THE LARVA AND DURING METAMORPHOSIS	425
V. THE INTRACELLULAR BACTERIA DURING THE ADULT STAGE	426
VI. DISTRIBUTION OF THE FREE BACTERIA IN THE ALIMENTARY TRACT OF THE ADULT	426
VII. THE RELATION BETWEEN CALANDRA AND ITS INTRACELLULAR BACTERIA	427
VIII. TRANSMISSION OF THE INTRACELLULAR BACTERIA FROM ONE GENERATION TO THE NEXT	429
(a) The Female Genital Organs and the 'Ovarian Bacterial Cells'	429
(b) Infection of the Egg	429
IX. THE BACTERIA DURING EMBRYONIC LIFE	430
(a) Formation of the 'Alimentary Bacterial Cell-mass'	430
(b) Formation of the Genital Rudiments and the Appearance of the 'Ovarian Bacterial Cells'	430
X. FORMATION OF THE OVARIOLES DURING METAMORPHOSIS	431
XI. MODE OF TRANSMISSION OF THE INTRACELLULAR BACTERIA IN OTHER INFECTED CURCULIONIDS	431
XII. SUMMARY	434
XIII. ACKNOWLEDGEMENTS	434
BIBLIOGRAPHY	435
EXPLANATION OF PLATES	435

1. INTRODUCTION.

IN my previous work on *Calandra* (1927) reference was made to the presence in the larval stage of a mass of cells between the nervous system and the alimentary canal in the region where the fore-gut passes into the mid-gut. On account of its

TEXT-FIG. 1.



Portion of a section through a mesenteric caecum showing bacteria, *b*, within the 'bacterial cells'. $\times 930$.

future anatomical relation to the mid-gut of the adult, this mass was referred to as the 'accessory cell-mass'.

More work on the subject revealed the fact that the cells of this mass contain in their cytoplasm numerous micro-organisms which proved to be bacteria (*b*, Text-fig. 1).

This mass of cells will therefore be referred to as the 'alimentary bacterial cell-mass', and its cells as the 'alimentary bacterial cells', in order to distinguish them from similar cells at the tips of the ovarioles to be described in the course of this paper and to be referred to as the 'ovarian bacterial cells'.

Pierantoni (1927) merely refers to the 'accessory cell-mass'

of the present author as an 'organo symbiotico' with no evidence concerning the exact relation between the micro-organisms contained within the cells of this organ and the weevil in question. His account seems to be based mainly on my previous work. He only describes the mass under consideration as being paired in the larva. For the mere fact that this mass is saddle-shaped in some sections certainly suggests a paired nature; but, when a whole series of sections is examined, there is not the slightest doubt that the cells in question are in one mass.

In this paper it is only proposed to give a preliminary account of the structure of these intracellular bacteria, their mode of transmission from one generation to the next, and their activity during the life of their host.

II. TECHNIQUE.

The developmental history of the bacterial cells was best studied in material fixed in Carl's fluid and stained in Delafield's haematoxylin (Mansour, 1927).

For the study of the bacteria in the cells and tissues of their host, Schaudinn's fluid proved the most suitable fixative. The organs to be studied were dissected out by means of very fine needles and transferred to the fixative for 3-5 minutes. They were then washed thoroughly in 50 and 70 per cent. alcohol, dehydrated, embedded, and sectioned in the usual way.

Smearing before fixation was also found useful for the study of the general structure of the bacteria. After drying up, the fixative was applied for about 5 minutes, then the smear was washed under the tap, dried, and stained.

Giemsa stain as used by Minchin¹ and Gram's as recommended by Eyre² gave very good results. Alkaline methylene blue was also found suitable for smear preparations.

¹ 'The Microtomists' Vade-Mecum', London, 1928.

² J. W. H. Eyre, 'Bacteriological Technique', Philadelphia and London, 1916.

III. THE INTRACELLULAR BACTERIA.

The intracellular bacteria are in the form of bacilli rounded at both ends and frequently joined together in strings, some being as much as 50μ long (Text-fig. 4). The separate bacilli are motile and vary from 3μ to 5μ in length and are about 0.6μ wide. They stain well with Giemsa, both in

TEXT-FIGS. 2-4.

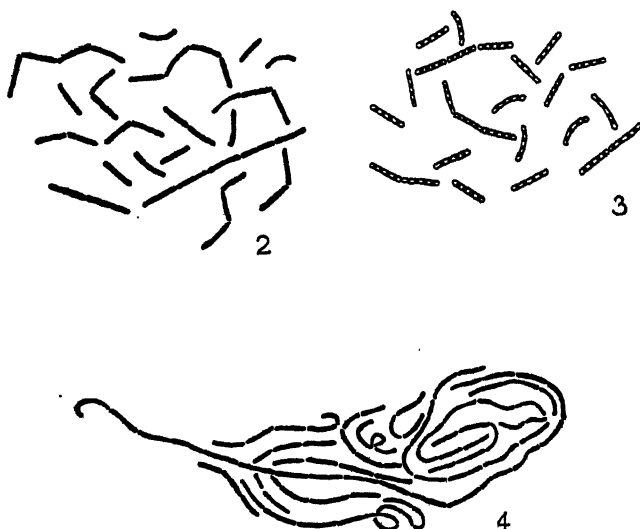


Fig. 2.—From a smear preparation of the mid-gut of an adult. Giemsa.

Fig. 3.—From a smear preparation of the mid-gut of an adult. Gram.

Fig. 4.—From a smear preparation of the 'alimentary bacterial cell-mass' of the larva. All $\times 2660$.

sections and smears, showing a uniform rod-like structure (Text-fig. 2). With Gram's, to which they are positive, they exhibit a distinct beaded structure (Text-fig. 3). Smears from the various stages of the host show that the strings are longer and more numerous in the egg, larva, and pupa: on the other hand, they are shorter and the presence of separate bacilli is more

frequent in the prepupal and adult stages. This is probably due to the fact that in the egg, larva, and pupa, the bacteria being comparatively inactive do not separate from their chains. In the prepupa, the breaking up of the chains into bacilli may be connected with the migration of the 'bacterial cells' to their final position around the developing mid-gut. Finally, in the adult, the bacteria themselves show great activity, and pass from their host-cells in the form of bacilli into the lumen of the alimentary tract (see p. 426).

In the larval stage the arrangement of the bacteria within the cells is very characteristic. Examination of smears shows that the long strings are coiled round one another in a peculiar fashion (Text-fig. 4). This appears to be due to the crowding of the strings within a limited space.

Artificial cultivation has not so far been attempted.

IV. THE 'ALIMENTARY BACTERIAL CELL-MASS' IN THE LARVA AND DURING METAMORPHOSIS.

The first conspicuous appearance of this mass, the mode in which it takes its place during the larval stage, its fate during metamorphosis, and the position of its cells in the adult, have been all described in the course of my previous paper.

Throughout the larval stage the bacteria within the cells are comparatively inactive and the 'mass' is to be found ventral to the gut and separated from the digestive epithelium by the muscular wall of the latter. Examination of the larva shows that the digestive epithelium and the food material inside the alimentary canal are free from the characteristic bacteria contained within the cells of the 'bacterial cell-mass'.

During metamorphosis the 'bacterial cells' are arranged round the developing mid-gut epithelium and ultimately form the outer walls of the anterior mesenteric caeca.

V. THE INTRACELLULAR BACTERIA DURING THE ADULT STAGE.

Contrary to their behaviour during the larval stage, the intracellular bacteria are very active during the imaginal life.

They pass in large compact ball-like masses into the lumena of the respective mesenteric caeca, and thence to the cavity of the mid-gut, where they infect some of the epithelial cells (fig. 1, Pl. 22). The bacteria in a newly infected cell grow actively and form a spherical mass (*m b*, fig. 2, Pl. 22) within the cytoplasm, which in this region stains more lightly than the rest of the cell. Eventually these masses pass into the lumen of the mid-gut (fig. 3, Pl. 22) in a similar fashion to that described for the original 'bacterial cells'.

VI. DISTRIBUTION OF THE FREE BACTERIA IN THE ALIMENTARY TRACT OF THE ADULT.

From the anterior portion of the lumen of the mid-gut the liberated bacteria which are in the form of bacilli spread anteriorly and posteriorly into the fore and hind guts respectively. In the gizzard they mix with the food of their host and apparently grow on it. Their number decreases gradually forwards. In the hind-gut they are present in large quantities mixed with the indigested food. In the anterior portion of this region of the gut they are very numerous indeed, but they become scarce posteriorly. The decrease in number of these bacilli seems to be correlated with the appearance of a coccus form; and in the crop and the hind portion of the proctodaeum where the bacillus form is very scarce, the coccus form is found in large quantities.

It is known that a bacterium may vary greatly under different conditions (Mercier (1907) and Eyre¹), and as the conditions within the cells are totally different from those in the lumen of the gut, it seems not unreasonable to suggest that the bacterium under consideration, when intracellular, is always rod-shaped, but when growing on the food of its host assumes after a short time a more or less rounded form.

Examination of the faeces of the adult supports this conclusion. It has been mentioned above that the bacillus form is

¹ Footnote, p. 423.

found in large quantities in the anterior half of the proctodaeum. In the faeces this form is practically absent, while the coccus form is very prevalent and forms a considerable portion of the faecal matter. This shows probably that by the time the indigested food is ready to be passed out, most of the bacilli have assumed the coccus form.

In the preparations examined no sign of disintegration or digestion of these bacteria has been observed. It is probable, therefore, that after being liberated from the cells, the bacteria live for a short time on the food taken in by their host and pass to the exterior mostly in the form of cocci.

VII. THE RELATION BETWEEN CALANDRA AND ITS INTRACELLULAR BACTERIA

Great importance has been attached recently to the presence of intracellular organisms in the alimentary tract of certain insects, especially in those with cellulose feeding habits. Buchner (1928 a), Pierantoni (1927), and others assume that such micro-organisms render valuable nutritive services to their hosts and describe the relation as being symbiotic. In the light of the present work there seems to be a doubt as to the accuracy of this assumed function of the micro-organisms.

The larva of *Calandra*, being inside the grain throughout its life, feeds entirely on the internal contents, i.e. starch and proteid-grains. It has been pointed out (p. 425) that the intracellular bacteria here are not found within the alimentary tract, and there seems to be no doubt whatsoever that the nutritive material the larva needs is digested with the aid of enzymes secreted by the digestive epithelial cells.

The adult, on the other hand, feeds inside the grain only for a very short period after its eclosion. It then bores its way out through the pericarp and the testa and lives the rest of its life outside the grain. Its food is similar to that taken in by the larva. It differs in the slight amount of cellulose eaten up during the emergence from the grain and again during the attack of

fresh material. This amount of ingested cellulose taken in is quite small and a very similar quantity has been observed in the faeces ; so that the question of cellulose digestion here is of minor importance. Thus the food of the adult is practically similar to that of the larva and consists mainly of starch and proteid-grains.

Uvarov (1929), in his masterly summary of the literature on ' Insect Nutrition and Metabolism ', has pointed out that proteases and diastases occur in the digestive fluids of all insects studied. It is quite probable that the larva of *Calandra*, too, possesses similar enzymes for digestion. As the mid-gut of the adult *Calandra* is ectodermal in origin as is that of the larva, it is quite conceivable that the digestion in the adult is similar to that in the larva without the help of the intracellular bacteria it harbours. This conclusion holds good unless it is to be assumed that the digestive epithelium of the adult lacks certain enzymes, or is supplemented by certain enzymes due to the activities of the bacteria. At present there is no evidence at all to support such an hypothesis.

Hylobius abietis, which contains similar ' bacterial cells ' (Mansour (1927) and Buchner (1928 a)), illustrates more clearly the doubt which the present author entertains concerning the supposed role of such intracellular bacteria. As in *Calandra*, the intracellular bacteria are comparatively inactive in the larval stage. During metamorphosis the ' bacterial cells ' arrange themselves round the developing digestive epithelium ; probably in the adult *Hylobius* they behave in a similar manner to that described for *Calandra*. The larva and adult feed on different food materials. The larva feeds inside the old pine stumps and takes in large quantities of wood. The adult, on the other hand, feeds on young pine shoots and needles and probably does not touch hard wood at all. If the intracellular bacteria in *Hylobius* help in the digestion of wood, as has been assumed by many authors, one would expect them to be active in the larval stage as well.

In conclusion, however, it must be admitted that the role of the bacteria in the gut of *Calandra* is obscure. What the

relation between the two organisms—bacteria and Calandra—may be, no one really knows. Under these circumstances, to describe the behaviour of the bacteria and Calandra as symbiotic is premature, misleading, and unjustifiable.

VIII. TRANSMISSION OF THE INTRACELLULAR BACTERIA FROM ONE GENERATION TO THE NEXT.

(a) The Female Genital Organs and the 'Ovarian Bacterial Cells'.

The infection of a new generation takes place in the ovarioles. There are two ovaries, each consisting of two acrotrophic ovarioles which open into a common oviduct. The two oviducts, one from either side, open into the uterus, which leads into the vagina. The anterior extremity of the latter is expanded to form the bursa copulatrix. Into the dorsal surface of the vagina, near its posterior end, opens a very narrow tube which leads into the horseshoe-shaped thickly chitinated receptaculum seminis.

Examination of an ovariole discloses the presence at its tip of a group of 'bacterial cells' similar to those surrounding the mesenteric caeca of the adult. These 'bacterial cells' are closely associated with the germarium and are enclosed within the delicate membrane investing the whole ovariole. In male gonads such 'bacterial cells' are absent.

(b) Infection of the Egg.

During oogenesis the bacteria leave their host-cells (fig. 4, Pl. 22) and pass backwards into the germarium, where they are to be found in the nutritive fluid scattered in between the developing oocytes and the nutritive cells. Growing eggs in the germarium are found to be infected (fig. 5, Pl. 22). In all the subsequent stages of growth of the ovarian egg similar infection has been observed in the cytoplasm (fig. 6, Pl. 22). Within the cytoplasm this infection remains and when the yolk is deposited the bacteria are to be found scattered in between the globules (fig. 7, Pl. 23). The bacteria remain throughout the early stages of embryonic development in a similar position.

In some females the genital ducts were found to contain practically pure cultures of similar bacteria; in all the males examined no bacteria were seen in the corresponding organs. Further, it has been observed that masses of bacteria pass down among the growing oocytes into the uterus. So the balance of evidence seems to point to the fact that the bacteria present in some female ducts come down from the tips of the ovarioles. It seems that owing to the large number of bacteria present in the region of growing eggs, the infection of the uterus is direct and not through the exterior genital aperture from the proctodaeum as Buchner (1928*b*) assumes for *Hylobius*, *Otiorrhynchus*, and the great majority of infected Curculionids.

The bacteria in the genital ducts of the female of *Calandra* take no part in the infection of the eggs.

IX. THE BACTERIA DURING EMBRYONIC LIFE.

(a) Formation of the 'Alimentary Bacterial Cell-mass'.

The formation of the 'alimentary bacterial cell-mass' ('accessory cell-mass') has been dealt with previously (Mansour, 1927). It need only be mentioned here that the change in the cytoplasm of the cells forming this mass is due to the invasion of bacteria from the surrounding yolk-mass.

The 'bacterial mass' in question appears in all developing eggs and is present in all larvae. It is destined to infect the alimentary canal of the adult.

(b) Formation of the Genital Rudiments and the Appearance of the 'Ovarian Bacterial Cells'.

The genital rudiments are paired. They are differentiated at a very early stage in the embryonic development. They first appear as a mass of cells situated at the posterior end of the egg in between the yolk-mass and the blastoderm. Towards the end of the embryonic life these rudiments are situated in the dorsal region of the posterior half of the embryo. They retain this relative position throughout the latter part of the embryonic period and the whole of the larval stage.

Examination of a number of genital rudiments in late embryos and larvae shows that two types can be distinguished, one associated with few bacterial cells (*ov b c*, fig. 9, Pl. 23), and from its developmental history giving rise to ovarioles, the other free from such cells and giving rise to testes (fig. 8, Pl. 23).

The rudiments destined to give rise to ovarioles (fig. 9, Pl. 23) show bilateral symmetry and each half consists of a small mass of 'bacterial cells' (*ov b c*), a mass of germ-cells (*fe g c*), and a few basal cells with deeply staining nuclei (*bs c*).

The mode of infection of the ovarian rudiments has not so far been followed in detail. Apparently some of the cells associated with these rudiments, while still close to the yolk-mass, are invaded by bacteria coming from the inner cytoplasm of the egg.

X. FORMATION OF THE OVARIOLES DURING METAMORPHOSIS.

Every ovarian rudiment gives rise to two ovarioles and an oviduct. The basal cells grow backwards in the form of a V-shaped tube, while the other constituents of the rudiment are separated into two equivalent sets, each set consisting of a mass of germ-cells and a group of 'bacterial cells'. At this stage the developing ovariole is club-shaped (fig. 10, Pl. 23). The germ-cells divide actively and block the cavity of the tube, whose walls become very thin and form the inner coat of the ovariole (*i c d o*, fig. 11, Pl. 23). The bacterial mass increases slightly in size and remains all the time at the anterior extremity. From the point of junction of the developing ovarioles, the oviduct grows backwards to meet the other genital ducts, which in the meantime have been developing inwards from the hypodermis, and by the time metamorphosis is completed, the oviducts are found continuous with the uterus.

XI. MODE OF TRANSMISSION OF THE INTRACELLULAR BACTERIA IN OTHER INFECTED CURCULIONIDS.

Masses similar in appearance and in behaviour during metamorphosis to the bacterial mass of *C. oryzae* have been described by the present author (1927) in *C. granaria* and *Hylobius abietis*. In the case of *C. granaria* it has been

ascertained that the mode of infection of the egg is similar in all respects to that described for *C. oryzae*.

Similar 'bacterial masses', probably behaving in a similar fashion, were also described by me in *Odioporus glabri-collis* and *Rhyncolus lignarius*.

Examination of the larvae of *Rhyncolus lignarius* discloses the fact that the genital rudiments are of two kinds, one with cells similar to those of the infected mass, and the other free from such cells. Probably further work would prove that the rudiments with infected cells give rise to ovarioles, and that the mode of infection is similar to that which has been described for *Calandra*.

Buchner (1928 *a*) independently confirms the present author's observations on *Hylobius*, and describes similar masses in *Otiorrhynchus inflatus*, *Pissodes notatus*, *Cryptorrhynchus lapathi*, *Cionus* sp., *Sibina pellucens*, *Protapion aeneum*, and *Cleonis* sp.

With regard to the mode of infection of the eggs of these Curculionids, Buchner describes two methods: one for the family Cleonidae, and the other for the rest of the infected Curculionids, with especial reference to *Hylobius abietis* and *Otiorrhynchus inflatus*.

With regard to the mode of infection in the Cleonidae, Buchner (1928 *a*, pp. 41-3) writes: 'So far I have found the counterpart of the fungus syringes in Siricidae, Cerambycidae, and Anobiidae only in the Cleonidae—it takes the form of bacterial syringes. At the place where the last segment joins the penultimate and which in the position of rest is withdrawn into the preceding segment, the narrow orifices of a long club-shaped organ lie on both sides. On crushing the syringe endless masses of slender symbiotic bacteria are squeezed out. We have not yet had the opportunity of observing the function of these syringes during oviposition, so that we cannot decide whether clumps of bacteria issuing from the orifices and sticking to the egg surface are eaten by the young larva, or whether the bacteria make their way in through the micropyle, and are thus introduced into the next generation much earlier.'

He describes and figures four large 'Bakterienorgane', apposed to the wall of the anterior portion of the mid-gut of *Cleonus*, but admits that in the adult he has not been able to demonstrate the presence of 'bacterial cells' along the mesenteron. Evidently these masses are not to be compared with those of *Calandra*, *Hylobius*, &c., and need not concern us here.

The case of *Hylobius* and *Otiorrhynchus*, on the other hand, raises important points.

With reference to the mode of transmission in these two insects, Buchner (in the same work, p. 44) mentions: 'So far, it is only in *Hylobius* and *Otiorrhynchus* that I can say that transference takes place in such a simple and ingenious way. Here I find, in the large bursa copulatrix, considerable quantities of bacteria near and between the sperm masses, and I assume that they enter the micropyle of the weevil's egg with the spermatozoa. Evidence for this must be sought by further difficult research.'

The same author (1928 b, p. 32) adds that in the great majority of weevils this is evidently the original mode of infection of the egg, and assumes that the bacteria in the genital ducts of the female come from the proctodaeum through the anus and the external genital aperture. He also mentions, without giving evidence for such a statement, that the ovarian eggs are not infected, and that the fate of these bacteria during embryonic development has not yet been determined.

It has been pointed out (p. 430) that similar bacteria are found free in the female genital ducts in the case of *Calandra* and that these organisms pass downwards from the tips of the ovarioles. It may be that the bacteria in the female genital ducts of *Hylobius* and *Otiorrhynchus* have a similar origin. As there is no evidence at all for Buchner's assumptions that the bacteria enter the egg with the sperms, it is probable that further work on *Hylobius* and *Otiorrhynchus* will prove that the mode of infection is similar to that which has been described for *Calandra*.

XII. SUMMARY.

1. The bacteria within the 'bacterial cells' of *Calandra* are in the form of bacilli.

2. The bacilli do not pass into the alimentary tract of the larva.

3. In the adult the bacilli pass from their host-cells into the lumen of the gut, mix with the food there, and pass out with the faeces mostly in the form of cocci.

4. The relation between *Calandra* and the intracellular bacteria is obscure and so far cannot be described as symbiotic.

5. 'Bacterial cells' have been found at the anterior tips of the ovarioles.

6. The ovarian eggs are invaded at a very early stage during their growth by bacteria coming from the 'ovarian bacterial cells'.

7. The bacteria remain in the cytoplasm of the egg scattered in between the yolk globules throughout the early embryonic life.

8. In all developing eggs the 'alimentary bacterial cell-mass' appears during the latter part of embryonic life.

9. In the eggs destined to give rise to females the genital rudiments are associated with 'bacterial cells'.

10. The developmental history of the 'ovarian bacterial cells' has been followed out.

XIII. ACKNOWLEDGEMENTS.

I desire to express my best thanks to Professor Victor Jollos (formerly of the Egyptian University) for his advice concerning technique.

I am further greatly indebted to Dr. E. J. Allen for kindly allowing me to finish this work at the Laboratories of the Plymouth Marine Biological Station.

BIBLIOGRAPHY.

- Buchner, P. (1928*a*).—‘Holznahrung und Symbiose.’ Berlin.
 — (1928*b*).—“Ergebnisse der Symbioseforschung”, ‘Ergebnisse der Biologie’, 4.
 Mansour, K. (1927).—“The Development of the larval and adult mid-gut of *Calandra oryzae* (Linn.)”, ‘Quart. Journ. Micr. Sci.’, 71.
 Mercier, L. (1907).—“Recherches sur les bacterioides des Blattides”, ‘Arch. f. Protistenkunde’, 9.
 Pierantoni, U. (1927).—“L’organo simbiotico nello sviluppo di *Calandra oryzae*”, ‘Rend. Acc. Sc. Fis. Mat. Napoli’, Series 3*a*, 35.
 Uvarov, B. P. (1929).—“Insect Nutrition and Metabolism”, ‘Trans. Ento. Soc. London’, 76.

EXPLANATION OF PLATES 22 AND 23.

All figures are from camera lucida drawings.

LIST OF REFERENCE LETTERS.

b, bacteria or bacterium; *bs c*, basal cells; *cy*, cytoplasm; *fc*, follicular cells; *fgc*, female germ-cells; *ftc*, fat cells; *icdo*, inner coat of the wall of developing ovariole; *magc*, male germ-cells; *mb*, mass of bacteria; *mg ep*, mid-gut epithelium; *mus w*, muscular wall; *n*, nucleus; *nl*, nucleolus; *ocdo*, outer coat of the wall of developing ovariole; *ovbc*, ‘ovarian bacterial cells’; *yg*, yolk-globule.

PLATE 22.

Fig. 1.—Portion of a transverse section through the anterior region of the mid-gut of an adult showing infected epithelial cells. Schaudinn and Giemsa. $\times 600$.

Fig. 2.—An epithelial cell with a mass of bacteria (*mb*) within the cytoplasm. Schaudinn and Giemsa. $\times 800$.

Fig. 3.—Portion of a transverse section through the anterior region of the mid-gut of an adult showing the passage of a mass of bacteria into the lumen of the gut. Schaudinn and Giemsa. $\times 800$.

Fig. 4.—Portion of a section through the anterior tip of an ovariole during oogenesis. Schaudinn and Giemsa. $\times 800$.

Fig. 5.—A section through a very young oocyte while still in the germarium, showing bacteria (*b*) already in the cytoplasm. Schaudinn and Giemsa. $\times 900$.

Fig. 6.—A section through an oocyte surrounded by follicular cells. The size at this stage is nearly half that of the fully grown egg. Schaudinn and Giemsa. $\times 480$.

PLATE 23.

Fig. 7.—A portion of a section through an almost fully grown egg, showing bacteria (*b*) in between the yolk-globules (*y g*). Schaudinn and Giemsa. $\times 900$.

Fig. 8.—Portion of a longitudinal section through a second instar larva, showing one male genital rudiment. Carl and Delafield's haematoxylin. $\times 400$.

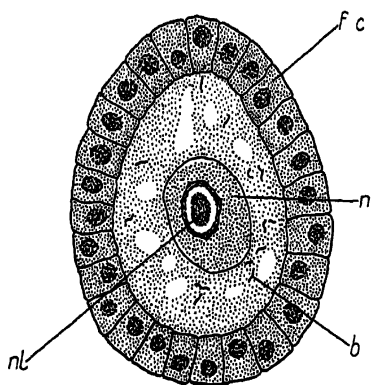
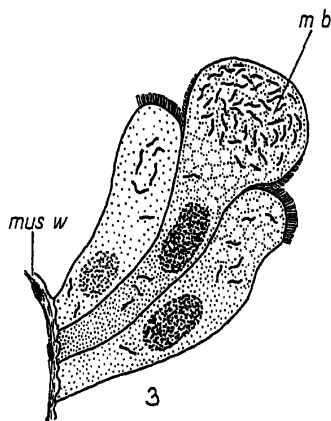
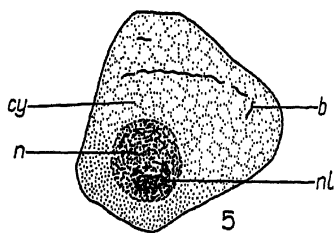
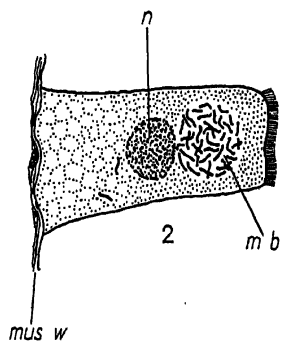
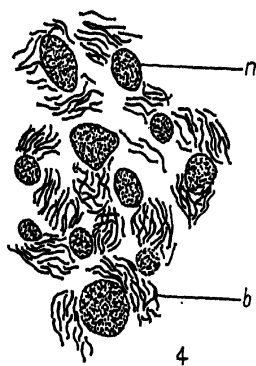
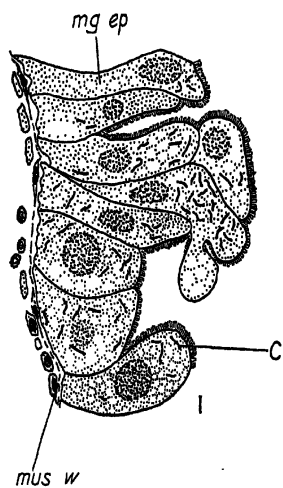
Fig. 9.—Portion of a longitudinal section through a second instar larva, showing one female genital rudiment with ovarian bacterial cells (*ov b c*). Carl and Delafield's haematoxylin. $\times 400$.

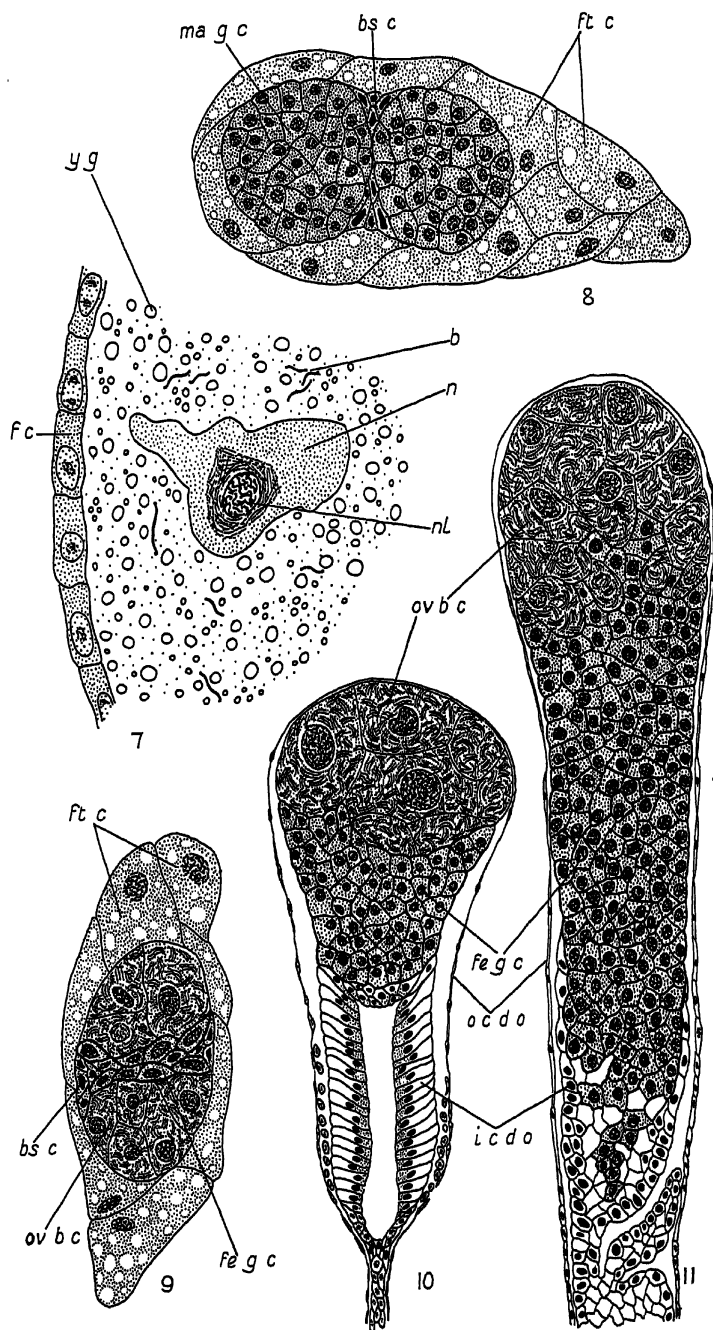
Fig. 10.—Portion of a longitudinal section through a prepupa, showing one developing ovariole with 'ovarian bacterial cells' (*ov b c*) at the anterior extremity. Carl and Delafield's haematoxylin. $\times 350$.

Fig. 11.—Portion of a longitudinal section through a pupa showing an ovariole at a stage later than that represented in the previous figure. Carl and Delafield's haematoxylin. $\times 350$.

APPENDIX.

Since this article was written, I have examined *Baris maculipennis*, a Curculionid which breeds in the fruits of *Citrellus colocynthis*. This weevil was found to harbour intracellular bacteria similar in their relative position to those of *Calandra* and *Hylobius*. The genital rudiments are of two kinds, one with and the other without bacterial cells. This supports what has been mentioned on p. 438 concerning the transmission of such intracellular bacteria in infected Curculionids.







Variation in the Histological Condition of the Thyroid Glands of Sheep with regard to Season, Sex, Age, and Locality.

By

Esther Lowe, M.Sc.,

Assistant Lecturer in Zoology, University of Manchester.

With Plates 24 and 25.

CONTENTS.

	PAGE
1. INTRODUCTION	437
2. MATERIAL	438
3. METHODS	440
4. INFLUENCE OF SEASON ON HISTOLOGICAL STRUCTURE	441
5. INFLUENCE OF AGE ON HISTOLOGICAL STRUCTURE	451
6. INFLUENCE OF LOCALITY ON HISTOLOGICAL STRUCTURE	455
7. INFLUENCE OF SEX ON HISTOLOGICAL STRUCTURE	456
8. INFLUENCE OF CASTRATION	459
9. INFLUENCE OF PREGNANCY	460
10. SUMMARY	461
REFERENCES	463
EXPLANATION OF PLATES	463

INTRODUCTION.

Our present knowledge of the thyroid gland has been derived chiefly from the investigations of workers on the human gland. A comparatively small amount of work has been done on the thyroids of the lower animals, and this has been chiefly of an experimental nature, very little attention having been paid to the histology.

In 1924, Mrs. Bisbee (Ruth C. Bamber) (1), Lecturer in Zoology at the University of Liverpool, put forward an interesting suggestion as to the possible connexion between Braxy and thyroid activity in sheep.

McCarrison (5, 1917) and others have demonstrated a seasonal prevalence of goitre in human beings, and Seidell and Fenger (10, 1912), by iodine estimations, demonstrated a seasonal variation in the activity of the thyroid glands of sheep, hogs, and cattle. It is very widely stated that a seasonal variation has been found in the glands of all animals investigated, but this statement appears to rest entirely on the work of Seidell and Fenger. Martin (8, 1912), however, working in the British Isles on sheep alone failed to find any definite seasonal variation; also it is by no means clear that Seidell and Fenger's results were not complicated by sex variations which certainly exist and which were not taken into account. It seems by no means established, therefore, that seasonal variation does occur especially in the sheep of this country.

In view of this uncertainty, Mrs. Bisbee suggested the present investigation into the activity of the thyroid glands of sheep, and its correlation with age, sex, season, and locality. Attention has also been paid to the influence of castration and pregnancy.

The sheeps' glands were obtained from the Liverpool Abattoir, and my sincere thanks are due to Mr. Johnstone, meat inspector, and to Mr. Roberts of Pritchard and Elsons, butchers, for their kindness in giving me every opportunity of obtaining material. The laboratory work was carried out in the Zoological Department of the University of Liverpool during the year October, 1924–October 1925, and I wish to express my thanks to Professor Dakin, Dr. Johnstone, and Mr. Horton of this University for their interest and suggestions during the course of the work. My especial thanks are due to Mrs. Bisbee for suggesting the work originally and for much valuable advice in regard both to the actual work itself and to the preparation of the paper.

MATERIAL.

The sheeps' thyroids used in this investigation were collected on Tuesdays or Wednesdays of each week so that the intervals between the successive samples were always regular. All the glands obtained were removed whole from freshly killed animals

and were in most cases placed in the fixative within 15–20 minutes after the death of the animal, so that the risk of post-mortem changes was as far as possible eliminated. The number of animals from which glands were taken varied from three to six per week according to the variety of material obtainable. When the sheep were all of the same age and sex, as was sometimes the case, it was not thought necessary to take more than three specimens, but more were taken on the occasions when a greater variety of material was obtainable. In all cases the approximate age of each sheep and the district from which it came were ascertained.

The main difficulty, however, connected with this investigation was the impossibility of obtaining sheep of all kinds at regular intervals throughout the year. A regular supply of glands from young mature, uncastrated males was obtainable from October until the end of May. Young mature castrated males were easily obtained from October to the beginning of May; but during the summer months from May onwards, when the spring lambs were put on the market, it became impossible to obtain anything but male and female lambs. Throughout the year, the thyroids of mature females have been the most difficult to obtain regularly. During the autumn and early winter months, from October to January, mature females of all ages were as readily obtained as males and wethers; but during the late winter and spring months, from January to May, not a single yearling female was obtained, although occasional aged females were put on the market. At the beginning of May the spring lambs were put on the market, but in addition to these, the supply of yearlings persisted throughout this month. These consisted of the yearling uncastrated males already mentioned and two yearling females which were obtainable on one occasion only. From the end of May onwards the glands of male and female lambs have been examined in about equal numbers to the complete exclusion of older sheep.

This irregularity in the supply of material has, of course, added greatly to the difficulty of obtaining a correct estimate of the seasonal and sexual variation in the histological structure

of gland. At the same time, however, it emphasizes the need for an investigation of this kind, since the records of the seasonal variation in the iodine content of sheeps' thyroids carried out by Seidell and Fenger in America and by Martin in the British Isles, were derived from composite samples and no attention was paid to age or sex. In the case of the American sheep, however, variations due to locality were eliminated by including in each sample the glands of sheep from all parts of the country.

METHODS.

The glands were in all cases cut transversely into pieces before being placed in the fixative. In cases where only one or two portions were taken from one gland, the middle of the gland was always used, as it was thought that this would give the best estimate of the general condition of the gland. In cases where an enlarged isthmus was present, a portion of this was also fixed and sectioned. In all cases complete transverse sections were taken either from the middle portion of the gland, as mentioned above, or from various parts.

During the course of this investigation many different fixatives have been used with varying degrees of success. Bouin's picro-formol was the first to be tried, but this was found to be quite useless for sheeps' glands; it turned the colloid into hard stony masses which powdered on the microtome knife and completely ruined the sections. Flemming's fixative, which is recommended by Guyer for thyroid, was also found to be a failure. The colloid became so horny in consistency that the masses would not cut but came out of the acini whole. Carnoy's and Gilson's fixatives were both tried, but they both produced a serious shrinkage of the colloid. Finally, 10 per cent. formalin was used and was found very satisfactory. It fixes both cells and colloid satisfactorily and does not cause shrinkage. This fixative, therefore, has been used throughout the present work.

The chief stains used have been Mann's methyl-blue-eosin and Mallory's triple stain. Iron haematoxylin has been used to a less extent.

THE THYROID GLAND OF THE SHEEP. INFLUENCE OF SEASON
ON HISTOLOGICAL STRUCTURE.

Up to the present time none of the numerous workers on the thyroid gland appear to have published any account of the effect of season on the histological structure. The minute structure of the thyroid has been studied in connexion with temperature ; also several workers have devoted themselves to a study of variation in the iodine content of the gland, which is of course correlated with histological changes ; but the correlation between season and histological structure as such, has not, to the writer's knowledge, received any serious attention.

It is known from McCarrison's (5, 1917) investigations that endemic goitre in man exhibits a definite seasonal prevalence. He states that in European countries goitre is supposed to originate most commonly in the months of March, April, May, and June. Also in parts of Himalayan India which are not reached by the monsoon, goitre tends to develop during the rainy season. Naturally the development of goitre involves drastic histological changes, but McCarrison does not discuss the correlation of season with any particular type of histological structure ; he accepts seasonal variation of iodine content from the work of Seidell and Fenger, and suggests that such seasonal variation in the iodine content of the gland may be correlated with the seasonal prevalence of goitre.

Seasonal investigations of the iodine content of the thyroid glands of cattle, sheep, and hogs were carried out by Seidell and Fenger in America, and similar investigations for sheep only were made by Martin in England. In both cases the results were obtained by analysis of composite samples of the glands from large numbers of animals. In both cases the investigations were undertaken not with a view to studying a seasonal variation, but in connexion with an effort to fix a standard for thyroid preparations used in medicine. It was therefore of no interest to them to ascertain the sex of the animals whose glands were used, and the variation found in the iodine content was attributed to the influence of seasonal changes without taking into

account the possibility of a seasonal variation in the proportions of the two sexes obtained. As it is well known that the iodine content, size, and physiological activity of the thyroid varies considerably with the sex of the animal, the predominance of one sex or the other amongst available material at different seasons of the year may have influenced considerably the results obtained. Be that as it may, the fact remains that the results obtained in England by Martin (8, 1912) differed widely from those obtained in America by Seidell and Fenger (10, 1912). Martin's results showed very little variation throughout the year. The lowest iodine content obtained was 0.3 per cent. of dry weight, and this minimum was obtained twice during the year, in the middle of November and again in the middle of April. The highest iodine content was 0.4 per cent. of dry weight, and was obtained in the middle of July. The estimate for October 14 was also nearly as high as that for July. Thus it is seen that the variation throughout the year in English sheep was less than 0.1 per cent. The results of Seidell and Fenger for American sheep exhibit a strong contrast to this. The figures obtained by them show a marked seasonal variation. The iodine content altogether is considerably lower than that of the English sheep, the highest figure obtained being 0.335 per cent. of dry weight on July 7, and the lowest, 0.048 per cent. and 0.042 per cent. obtained respectively on March 8 and May 26. As may be seen from the graph made by Seidell and Fenger (10, 1912), the curve drawn from the average results reaches its highest point early in November, and is at its lowest about April 14, and the figures show that the average iodine content for the months June to December is roughly three times that for December to May. It was also found that the weight of the gland varied in inverse proportion to the amount of iodine it contained, the average weight of the sheep's thyroid for summer and fall being 5.5 gm. and that for winter and spring 8.5 gm. A similar seasonal variation was also found in the iodine contents of the thyroid glands of cattle and hogs. In the case of the hog the maximum was reached early in September and the minimum early in March, whereas the maximum for cattle

occurred early in October and the minimum early in February.

The present investigation has been devoted to the study of the histological structure alone, and this has been used as an indication of the activity of the glands. The iodine contents have not been estimated, but the weights of the glands have been recorded for many weeks in succession at different periods of the year.

The facts with regard to seasonal variation have been deduced chiefly from a study of the glands of young uncastrated males aged from 3 to 16 months, although both castrated males and females have been obtained over sufficiently long periods to give significant data. The uncastrated males include: (1) sheep, aged from 8 to 15 months, obtained during the period October–April (inclusive); (2) lambs, aged 3 months, and sheep aged 15–16 months, during May; and (3), lambs, aged from 4 to 7 months, during the period June–August (inclusive).

It is fully realized that differences of age may have affected this account of seasonal variation to some extent. The results, however, do not seem to favour this conclusion, and since sheep are only born at one period of the year, viz. during January and February, it was impossible to obviate this difficulty.

For convenience the results will be discussed in this section under the periods October–December, January–May, and May–August.

October–December (inclusive).

The investigations were begun early in October and for the first three months, October, November, and December, all the glands obtained from first-year males, both castrated and uncastrated, were found to be in a fairly uniform condition. Practically all the sheep obtained during this period were from North Wales, from the area including Ruthin, Mold, Tallycefn, Abergele, and Trefnant, the latter place being the most common source of supply. There were, however, two uncastrated males which came from Yorkshire and whose glands were obtained on October 14. The glands of these two males formed the only

two exceptions to the account given below for the period October-December and will be discussed later.

The glands of all the other uncastrated males, and most of the castrated ones (wethers), contained a very large amount of colloid (figs. 1 and 3, Pl. 24). The acini were greatly distended, until in many cases the epithelial cells were flattened and in some examples almost obliterated. There were, however, various intermediate stages in which the epithelium was of a low cuboidal character. The glands as a whole were characterized by complete absence of new secretion. There was a strong tendency to thickening of the intervesicular parenchymatous tissue accompanied by an increase in the number of fibroblasts. In many cases there was considerable evidence that a previous hypertrophic process had been subjected to some influence which had caused it to revert to the colloid state forming the so-called 'colloid goitre'. For instance, the colloid in many cases contained a considerable amount of cell debris, and, in the larger acini, plications of the lining epithelium were found.

In many cases these glands possessed a large well-developed isthmus whose structure also exhibited characters of a colloid goitre and which was frequently the seat of a tumour which will be described in a later paper.

The most advanced case of goitre was obtained on November 18 from a wether aged about 10 months. In this gland the parenchyma had become greatly thickened, but there was a varying amount of colloid and some of the acini were quite large. The parenchyma was opaque and appeared as an almost structureless mass containing an increased number of fibroblasts. The epithelial cells were mostly cuboidal in shape and there was evidence of a process of cell division the products of which had broken down and been cast into the colloid. The condition was also complicated by the presence of the tumour mentioned above.

The only gland which showed any noticeable amount of new secretion during this period was that of a wether obtained on December 2. This gland agreed with all the others in that its

acini were greatly distended with large amounts of colloid. It also showed an extensive development of the fibrous stroma between the acini. Sprig-like projections from the acinar walls and a considerable amount of cell debris in the colloid showed it to be a gland which had undergone a period of hypertrophy. In all these characters it agreed with all other glands obtained during the same period, but it differed in that its epithelium was cuboidal instead of flat and in that large amounts of new secretion were still being poured into the already distended acini. McCarrison (6, 1925) records a similar condition arising in the glands of young animals under experimental conditions. He describes it as 'hypertrophic goitre, a balanced exaggeration of all the normal features of the gland's cycle of functional activity'. Only one other similar case has been found during the year, and that was the gland of a castrated yearling obtained on May 18.

It is perhaps necessary to mention here that the glands of young females which were obtained regularly during the period October–December inclusive differed widely in their histological structure from those of the uncastrated males and wethers obtained during the same period. With one exception, which was the last of the series, they were all in a state of active secretion (fig. 2, Pl. 24). It is not, however, proposed to discuss these in detail here as they will be dealt with later, in the section devoted to sex. The influence of age on the histological structure of the gland will also be discussed in a separate section.

The weights of all the thyroids used during the months October–December were recorded and compared. The average weight of the glands of the first-year uncastrated males during this period was 2.84 gm. Those of castrated animals of the same age averaged 1.72 gm. and of females 1.98 gm.

January–early May.

The glands of the yearling uncastrated males and wethers during the following four months, January, February, March, and April were not nearly as uniform in character as those

obtained during the period October–December. In early January the colloid condition characteristic of the autumn months tended to persist, but later in the month some of the males showed a more active condition of the gland. For instance, two males and a wether obtained on January 20 all showed signs of a return to the active condition. In one of the males the gland showed a considerable degree of hypertrophy. The acini contained a variable amount of colloid and the epithelial cells lining the acini were vacuolated and varied from a cuboidal to a columnar condition. One or two large acini in which the colloid condition still persisted were present; but on the whole the gland showed evidence of extreme activity. There was a plentiful supply of new secretion with a reduction in the amount of stainable colloid. The fibrous stroma was also well developed.

The thyroid of the wether obtained on the same day showed an intermediate condition, the middle of the gland being more or less in the colloid condition and the outer regions being more active. This arrangement was an almost invariable rule in glands in which two different conditions coexisted; the more centrally situated acini were always the less active. All the glands obtained in the following week, i.e. the last week in January, were in the colloid condition and showed no signs of recent activity. The gland of the one wether obtained was distinctly goitrous in condition. It contained smaller amounts of colloid than those of the males taken on the same date, but the connective tissue was very much thicker and showed an extensive development of fibroblasts.

The glands of all the yearling uncastrated males obtained during February, March, and April showed signs of increased activity (fig. 6, Pl. 24), in fact the colloid condition was not met with again in uncastrated male yearlings until May 18. The degree of activity in the glands during February, March, and April varied considerably, but all contained a fair amount of new secretion. The acini were more or less reduced in size, and the epithelial cells, instead of being flat, varied in shape from high cuboidal to high columnar and became vacuolated.

This increase of activity in the glands was accompanied by a marked distension of the capillaries.

One or two extreme cases were obtained on February 4 and 18 in which the increased activity of the gland had caused hypertrophy. The epithelium in these cases was high columnar and showed evidence of active cell division. The acini were reduced in size and many of them were irregular in shape owing to plications of the lining epithelium.

The glands of the castrated animals (wethers) during the same period, January-May 18, also showed a tendency to increased activity (figs. 7 and 9, Pl. 25), but they were not as consistent as those of the uncastrated males, and although the majority were in an actively secreting condition, colloid glands without a trace of new secretion occurred sporadically throughout the period. One of these colloid glands was obtained on February 4, those of the uncastrated males obtained on the same date being in an active condition. Another was obtained on March 5, and was one of three glands taken from wethers of the same age and from the same district, the other two being in a very actively secreting condition. Two more were from sheep killed on April 22. One of the glands weighed 6.01 gm. and was the heaviest gland obtained during the year. The other gland appeared to be in an inert condition with small acini and no new secretion.

From April 22 to May 10 the glands of all the yearling wethers were secreting actively and all showed a tendency to hypertrophy (figs. 7 and 9, Pl. 25). One gland obtained from a wether on May 18 was in the colloid condition, but as this was the last date on which wethers were obtainable, it was impossible to carry this line of investigation any further. This was disappointing because, in the case of the uncastrated males, the beginning of May proved to be another turning-point in the seasonal variation.

No glands were weighed during the spring months until March 11. From this date onwards the weights were recorded consecutively for castrated males alone. The average weight for castrated yearlings during the period March 11 to May 18

was 2.96 gm. This was considerably heavier than the average for the autumn and winter months. It was one of the glands during this period which weighed 6.01 gm. and, as noted above, was the heaviest gland obtained during the year.

Early May—middle of August.

From May 6 onwards male and female lambs aged 3 months and over were obtainable, the males amongst the spring lambs being in all cases uncastrated. In addition to these the supply of yearling uncastrated males persisted throughout May, and on one occasion mature females were obtained. This was fortunate because the condition of the glands of these yearlings proved that the renewal of the colloid condition of the gland found in the younger lambs at this period was a definite seasonal change and was not due entirely to the difference in the age of the animals whose glands were used from the beginning of May onwards.

On May 13 the glands of three uncastrated yearling males were obtained, two of which were in the colloid condition, while the third was very actively secreting (fig. 10, Pl. 25). On May 27 the glands from four yearlings, two uncastrated males and two females, were obtained, and all were found to be in the colloid condition (figs. 11 and 12, Pl. 25). The gland of one of the females was a diffuse colloid goitre, the amount of colloid being so great that some of it had forced its way out of the acini and lymph channels and had formed large translucent globules in the gland, which were clearly visible to the naked eye. Evidences of previous hypertrophy were present in the gland (fig. 11, Pl. 25).

The first spring lambs, aged from 2 to 3 months, were obtained on May 6, and an examination of their glands showed that these were also in the colloid condition, with greatly distended acini lined by flat epithelium. On May 20 the glands of five spring lambs, two females and three males, were obtained, and on this occasion it was found that the glands from the females were actively secreting, whereas those of all the males were in the colloid condition. One of the female thyroids was very active

and exhibited a certain amount of hypertrophy, but the other was active in certain areas only, the rest of the gland being in the colloid condition with acini of moderate size lined by flat epithelium.

From the beginning of May until the middle of August the glands of all the male lambs were found to be in the colloid condition, and many of them showed evidence of some degree of previous hypertrophy.

The glands of female lambs examined during the same period were not as uniform in appearance as those of the males. All contained large acini, but the amount of new secretion varied considerably. In some cases the epithelium was cuboidal and new secretion was entirely absent. In the cases in which the gland was producing new secretion, this activity was not as a rule associated with any diminution in the amount of older colloid. Many of the glands had cell debris in the colloid and the acinar walls were several cells thick, suggesting previous hypertrophy. Some of the glands were of a mixed character, the more centrally situated acini being in the colloid condition, while the outer areas of the gland were more actively secreting.

All the glands obtained between May 6 and July 8 were weighed and the averages calculated. It was found that in the cases of lambs the glands of females were heavier than those of males, the average weight for male spring lambs being 2.87 gm. and that for females 2.43 gm. The average weight for yearlings during May was greater than that for lambs, the average weight for uncastrated males being 3.78 gm. and that for females 2.55 gm. A comparison of these weights with those obtained for the autumn and early winter months shows that the glands of both sexes are heavier during May and June than during October, November, and December. The increase is obviously not due entirely to a difference in age as the glands of 4-month-old lambs in June were heavier than those of 9- or 10-month-old lambs in November and December.

A summary of the results obtained shows that there is a definite seasonal variation in the condition of the glands of young uncastrated males. The colloid condition of the gland

which prevailed during the autumn and early winter months was replaced at the end of January by a more active condition which continued until May, when it gave way once more to the colloid condition. The glands of castrated animals were only obtainable during the period October to May, but during this time their condition was more or less in agreement with that of the uncastrated males. They showed the same seasonal variation, but during the spring months when the glands of the young uncastrated males were all actively secreting, those of the wethers were not as uniform in condition and a few colloid glands were obtained. The glands of young females were only obtainable at two seasons of the year, viz. the autumn and again during the summer months May to August,¹ but a striking difference was observed in the glands obtained during these two periods. During the autumn the glands were all actively secreting and showed a reduction in the amount of old colloid accompanied by a tendency to hypertrophy. During the summer months, on the other hand, the glands contained very large amounts of colloid and the acini were greatly distended. In fact the glands of females, both spring lambs and yearlings, during this period, differed very little in condition from those of the males. The main difference observed was that the epithelium was not as flat in the females as in the males, and many of the female glands were producing a certain amount of new secretion in spite of the presence of large amounts of colloid.

It is of interest to notice in this connexion that the return of the glands of uncastrated male sheep to the colloid condition at the beginning of May coincided with a great increase of temperature which took place throughout the British Isles at that time. From the experiments of Mills (9, 1918) with the glands of guinea-pigs, it is known that an increase of temperature induces the colloid condition, whereas a decrease of temperature causes increased activity. This would provide a satisfactory explana-

¹ The glands obtained during the autumn were from mature females aged 8-11 months. Those obtained during the summer months were from lambs aged 3-7 months, with the exception of two which were obtained in May from mature females.

tion of the prevalence of the colloid condition in the glands of both sexes during the summer, but it does not explain the occurrence of the colloid condition in the glands of all the Welsh males during the autumn and early winter months, for this period included some of the coldest days of the year. The active condition of the glands of female sheep during this period does agree with the observations of Mills for guinea-pigs, but in the case of males, at any rate, the seasonal variation does not seem to depend entirely on changes of temperature.

INFLUENCE OF AGE ON HISTOLOGICAL STRUCTURE.

Most of the evidence in regard to the effect of age on the histological condition of the thyroid seems to have been derived from the study of the human thyroid, and the accounts given by the various workers are not entirely in agreement; for instance, McCarrison (5, 1917) states that after the age of 40 in human beings the gland tends to atrophy, the epithelium becomes less active, and its colloid and iodine content decreased. In opposition to this we have the account given by Vincent (12, 1922), who states that the maximum iodine content in the thyroid of man is found between the ages of 40 and 60 years.

In a later paper McCarrison (6, 1925) classifies goitre into three types and states that the incidence of acquired hyperplastic or parenchymatous goitre is low in early childhood, but increases gradually with advancing years; whereas colloid goitre has its highest incidence in early life, tending to disappear after full maturity has been attained. The remaining type, the hypertrophic goitre, described as an early stage of the parenchymatous goitre, tends to arise in young animals under insanitary conditions. Marine and Lenhart (7, 1909), on the other hand, state that the colloid condition occurs in older animals and active hyperplasias in young animals.

Nothing is known about the effect of age on the histological structure of the sheep's thyroid, but in 1913 Sutherland Simpson (11) published an account of some interesting experiments which he carried out on the relation of age to the effects of

thyroidectomy in sheep. It was found that the removal of the thyroids and internal parathyroids from 2-year-old sheep had no deleterious effects on the animals provided they were kept under hygienic conditions. The same operation was performed on 6-7-month-old lambs and they also suffered no ill-effects, but 2-month-old lambs developed into typical cretins as a result of the operation, and lambs aged from 5 to 7 weeks developed tetany and died. These experiments suggest that the thyroid is a much more important organ in the young lamb than it is in the adult sheep.

In the present paper an attempt has been made to correlate age with thyroid activity. The results so far discussed in connexion with seasonal variation have included only the young sheep aged from about 4 to 16 months, but in addition to these older animals were obtained periodically until May 6. Uncastrated males, females, and wethers, all older than 21 months, were obtainable on various occasions, but not in sufficient numbers nor at sufficiently regular intervals to enable one to form any estimate of a seasonal variation in their thyroids; the ages of these sheep ranged from 1 year 9 months to 4 years and over.

From the foregoing section it is clear that in all young animals the thyroid goes through alternate periods of activity and rest, and that these changes are correlated with season. In older animals, on the other hand, there seem to be no such obvious seasonal changes.

During October and November the glands of four old sheep were obtained, two from wethers aged 1 year and 9 months and two from 4-year-old females. In contrast to the glands of the younger animals obtained during the same period, none of these four glands was completely in the colloid condition. In the glands from the two castrated males the acini were very irregular in size, some containing a moderate amount of colloid, others practically none. There was a small amount of new secretion and the epithelial cells were more or less cuboidal. The connective tissue was thickened and one gland was distinctly goitrous in character. Both glands contained tumours,

one small and the other large. The glands of the females were less goitrous than those of the wethers and contained rather more colloid. The connective tissue was not abnormally thickened and the epithelial cells varied from a cuboidal to a low columnar shape. The amount of new secretion in both cases was small, but groups of cells were present in many of the acinar walls, particularly in the outer regions of the gland where the acini were small. The heavier of the female glands contained a large tumour.

During January and February the glands of seven older animals were examined. On January 13 the glands of three castrated animals of different ages were obtained. The ages were 2 years, 2 years and 9 months, and 3 years respectively. The gland of the 2-year-old animal was in the colloid condition with greatly distended acini and flattened epithelial cells. It was practically devoid of new secretion and contained a large tumour. The gland of the animal aged 2 years and 9 months was also in the colloid condition, but the epithelium in this case was low cuboidal instead of flat and there was no tumour. In some parts of the gland there was evidence of previous active cell division. The gland of the 3-year-old animal was in a condition of active hypertrophy. The size of the acini was variable throughout the gland. In some areas colloid was almost absent; and the epithelial cells in most areas were in a state of active cell division.

February 10 was the next date on which glands were obtained from aged animals. On this date the glands of four females, three of which were pregnant, were obtained. All were found to be in the colloid condition with flat acinar epithelium and no new secretion. The connective tissue was greatly thickened in all four cases, but was more opaque and goitrous in appearance in the pregnant than in the non-pregnant animals (cf. figs. 4 and 5, Pl. 24). In the latter the intervesicular parenchymatous cells were not as completely atrophied as in the pregnant females. The glands of the pregnant females all contained tumours. No more glands from aged animals were obtained after this date until April 29, when glands were taken from an

aged female and a 3-year-old wether. That of the female was in the colloid condition throughout. The epithelium was completely flattened and the acini were fairly regular in size and shape and all greatly distended with colloid. Traces of previous hypertrophy were present in the form of localized thickenings of the connective tissue. The gland of the 3-year-old wether (fig. 8, Pl. 25) was also mainly in the colloid condition with signs of recent hypertrophy in the form of infolded acinar walls and areas of cell multiplication. In one area the hyperplastic condition still persisted. The epithelium throughout was mainly low cuboidal. In the following week the last of the series of glands from aged animals was obtained. This was from a very old male whose age could not be definitely determined. The gland was one of the heaviest obtained, its weight being 5.12 gm. It was in the colloid condition throughout with greatly distended acini of irregular size. The intervesicular tissue was greatly thickened and invaded by fibroblasts. In many of the larger acini the epithelium was plicated in places and the colloid contained cell debris from an earlier hyperplastic condition of the gland. A very large tumour was present.

Viewed as a whole, these results seem to point to the conclusion that the colloid state is the one most commonly found in the glands of old sheep, irrespective of sex or season, although it is fully realized that the glands examined were too few to admit of any but the most tentative of generalizations. The fact, however, that with one exception all the glands of aged animals obtained between January 13 and May 6 were in the colloid condition, whereas during this period those of the young animals obtained were found to be actively secreting, does suggest that the apparently permanent colloid condition is directly related to the advanced age of the animals. The colloid condition in young animals after its disappearance in early January did not appear again until the beginning of May.

With increasing age, therefore, the gland appears to lose its power of response to environmental changes.

INFLUENCE OF LOCALITY ON HISTOLOGICAL STRUCTURE.

Most of the sheep used in this investigation have been obtained from North Wales, from the area including Denbigh, Ruthin, Mold, Abergele, Wrexham, Corwen, and Trefnant. The only exceptions were three sheep (two males and one female), obtained from Yorkshire, and eleven spring lambs from Ireland. The exact district from which the Irish lambs came was not known.

Mention has already been made of the fact that the glands of the Yorkshire sheep differed from those of the Welsh sheep obtained during the autumn months. Whereas the glands of the Welsh uncastrated males during this period were all in the colloid condition with a strong goitrous tendency, those of the two uncastrated males from Yorkshire were in a much less extreme condition. The acini were very varied in size, but none of them were as large as those of the Welsh males. Also there was a certain amount of new secretion and in some regions the capillaries were somewhat dilated. In fact, both showed a moderate amount of activity. The gland of the Yorkshire female was in a similar condition to that of the males and was not as active as those of Welsh females. None of the glands showed evidence of hypertrophy. It is of course impossible to draw any conclusion on the evidence of three glands, but their condition is recorded because they showed an interesting variation from the general rule, which was possibly due to a difference of locality.

The only other exceptional gland obtained during the same period was that of a Welsh female obtained on December 9 from Abergele. This gland, instead of being in the actively secreting condition which was characteristic of all other females of the same age, was in the colloid condition, with distended acini lined by flat epithelium and without any trace of new secretion. It showed no signs of previous activity. The condition of this gland is interesting in view of the fact that this was the only occasion on which sheep were obtained from a place near the sea-coast, and it is known from the records of various workers

on the thyroid gland that there is a higher iodine content and less activity in the glands of animals living near the sea-coast, due probably to an increase in atmospheric iodine. Hunter and Simpson (4, 1914) estimated the iodine content of sheep pastured in the Orkney Islands and found that the glands contained a very high percentage of iodine, in some cases about three times the normal amount.

The glands of the Irish lambs obtained during June and July showed no difference in either sex from those of the Welsh lambs obtained at the same time. As the part of Ireland from which they came was not ascertained, it was impossible to say whether they had pastured in a similar environment to that of the Welsh lambs or whether the similarity was due to the fact that the seasonal influence in this case was stronger than any local influence.

INFLUENCE OF SEX ON HISTOLOGICAL STRUCTURE.

It is well known from the work of McCarrison (5, 1917) that in man the thyroid gland of the female is larger, more active, and contains more iodine per unit of body-weight than that of the male. Fenger (3, 1914) also states that in cattle the females contain more thyroid tissue and more iodine therein per unit of body-weight than the males. It is also known that after sexual maturity is attained in human beings endemic goitre is more common in females than in males, although up to the age of puberty it affects both sexes equally.

The present investigations have shown that in sheep there is a very definite difference in the condition of the gland in the two sexes during the months October, November, and December (cf. figs. 1 and 2, Pl. 24). All the glands obtained from yearling uncastrated males during this period were in the colloid condition, with the exception of two, which were from a different district, but the glands of yearling females during the same three months were all in a very actively secreting condition. The acini were diminished in size and there was a reduction in the amount of old colloid. The epithelium lining the acini was columnar, whereas in the males it was flat. In practically all the females

it showed some degree of hypertrophy either in the form of cell multiplication or by the folding of the epithelium into the colloid in some of the acini. The only gland whose condition did not conform to the description given above was that of the female obtained on December 9 from Abergele. This gland has already been discussed in the section on the influence of district. Its condition resembled that of the males obtained on the same date much more than any of the females obtained during the same period.

In addition to the difference in structure of the glands of the two sexes, there was also a difference in the average weights, that for yearling uncastrated males being 2.34 gm. as compared with 1.98 gm. for yearling females.

The failure of the supply of thyroids of young females during the late winter and spring months made it impossible to estimate the sex difference during this period. It has already been stated in the section dealing with the effects of age that there was no sex difference in the glands of older animals during this period.

During the summer months, May, June, July, and August, females were again obtainable, although with the exception of those obtained from yearlings on May 27, all the glands examined during this period were from young lambs aged from 4 to 7 months and which therefore were not sexually mature.

The glands of the four yearlings, two uncastrated males and two females, obtained on May 27, have already been described in the section dealing with the effects of season on histological structure. Portions of two of them are represented in figs. 11 and 12, Pl. 25. All were in the colloid condition and showed evidence of having previously undergone a period of activity. This was particularly evident in the case of one of the females. This gland, which was the lightest of the four, exhibited an extreme condition of diffuse colloid goitre. The acini and lymph channels were greatly distended, and in addition much of the colloid had escaped and was lying free in the gland, enclosed only by the surrounding connective tissue capsule, and when the gland was cut it poured out as a viscous fluid. An examination of the sections showed that throughout the gland the acini

were invaded by plications of the lining epithelium whose cells were flattened. Cell-masses were present in the epithelium of some of the acini and the colloid contained desquamated masses of cell debris which had been cut off from the lining epithelium. The cells were not uniformly flat throughout the gland, although this condition was most common, but in some of the very large acini the cells retained their columnar shape in spite of the amount of colloid present, and some of them were still producing globules of new secretion.

The gland of the other yearling female was also in the colloid state, but its condition was not nearly so extreme. The colloid condition was most pronounced in the middle of the gland, tending to give way to a more active state in the outer regions. The glands of the two uncastrated male yearlings were purely colloid in condition (fig. 12, Pl. 25), and showed no trace of new secretion. The acinar epithelium of both showed evidence of previous activity in some parts of the glands.

The glands of the males were distinctly heavier than those of the females. They weighed 4.11 gm. and 3.29 gm. respectively, whereas the females only weighed 2.37 gm. and 2.73 gm.

The glands of the male and female lambs obtained during May, June, July, and August were also with very few exceptions in the colloid condition, but whereas those of the males were all pure colloid glands throughout and contained practically no new secretion, most of the glands from females were producing a certain amount of new secretion in some parts if not throughout, in spite of the fact that the acini were quite as large and contained as much colloid as those of the males. The acinar epithelium was flat in the glands of most of the males, but in the females it was more often cuboidal, although a certain amount of variation occurred in both. This difference in the condition of the acinar epithelium was the only difference observed in the glands of the two sexes during the summer months, but as the animals from which the glands were obtained were not sexually mature, one would perhaps scarcely expect to find a very striking difference between the sexes. The glands of the very few yearlings obtained during May agreed with those

of the lambs in showing very little difference between the sexes, but as only two females and five males were obtained it is impossible to draw any reliable conclusions.

The average weight of the thyroid of female lambs for the four months under discussion was 2.56 gm. as compared with 2.87 gm. for males. The two female yearlings averaged 2.55 gm. and the five males 3.78 gm.

Thus it is clear that a marked difference in the histological structure of the glands of the two sexes was found only during the autumn and early winter months. The condition of the females during the spring months was not recorded, but during the summer months very little difference was found in the glands of the two sexes, either in spring lambs or in yearlings.

INFLUENCE OF CASTRATION.

Practically nothing is known of the effect of castration on the human thyroid ; but Fenger (3, 1914), in his investigation of the iodine content of the thyroid gland of cattle, found that castrated males contain less thyroid tissue than either uncastrated males or females, and that the iodine content per unit of body-weight is intermediate between that of the uncastrated male and the female.

With regard to the amount of thyroid tissue in castrated animals, the present results are entirely in agreement with those of Fenger. All the thyroids obtained during the months October, November, and December were weighed and the averages calculated. It was found that the thyroids of the castrated animals were lighter than those of either the males or the females, averaging only 1.72 gm., whereas the glands of females averaged 1.92 gm. and those of the males 2.34 gm. During the spring months only a proportion of the glands obtained each week were weighed, but these showed a similar difference in the average weights of castrated and uncastrated males. The average for the uncastrated males was 3.58 gm., and that for the wethers, 2.86 gm.

With regard to the histological structure, the glands of wethers in most cases seemed to follow fairly closely the

seasonal variations observed in the glands of uncastrated males, except that during the early spring months, when the glands of all the males changed from the colloid to the actively secreting condition, the glands of the wethers were much more varied in their condition. Some became actively secreting, like those of the uncastrated males, while others remained in the colloid condition.

INFLUENCE OF PREGNANCY.

Most of the workers on the human thyroid state that an enlargement of the gland usually occurs during pregnancy. McCarrison has observed that goitres commonly originate during pregnancy and that enlargement of previously existing goitres is particularly liable to occur during this period.

Blair Bell (2, 1920) states that thyroid enlargements during pregnancy are due to storage of colloid.

Very little is known about the effects of pregnancy on the thyroids of the lower animals, but Fenger, from his investigations on the thyroids of cattle, finds that there is no apparent difference in size and physiological activity between glands from pregnant and non-pregnant females.

During the present investigation glands from pregnant females were only available on one occasion. The glands of three aged pregnant females were obtained on February 10, and on the same date the gland of another non-pregnant aged female from the same district was also taken. All the glands were found to be in a condition of colloid goitre with thickened inter-vesicular tissue and abundant evidence of previous hypertrophy (figs. 4 and 5, Pl. 24). Those of the pregnant females, however, were more goitrous in structure than the gland of the non-pregnant animal. In the latter the cells of the intervesicular tissue were still distinguishable, whereas in the glands of the pregnant animals many of the cells appeared to have atrophied. The glands of the pregnant animals weighed 3.42 gm., 2.67 gm., and 2.6 gm. respectively. They were all heavier than the gland of the non-pregnant female, which weighed only 2.14 gm.

This is of course very slight evidence, but as far as it goes it

is in keeping with the findings of McCarrison and Blair Bell for human beings, that during pregnancy the thyroid is enlarged.

SUMMARY OF FACTS REGARDING VARIATION IN SHEEPS' THYROID.

Influence of Season.

1. Uncastrated Males (yearlings and lambs). The glands of uncastrated males, aged from 8 to 12 months, were all in the colloid condition from October to the middle of January. From February to early May, glands from uncastrated males aged from 12 to 15 months were in the active condition. During May the glands from a few uncastrated males, aged 15-16 months, showed a return to the colloid condition. From the beginning of May to August the glands from male lambs, aged 3-7 months, were all in the colloid condition. Many of these showed evidences of previous hypertrophy.

2. Castrated Males (yearlings). The condition of the glands of castrated males was in agreement with that of uncastrated males during the autumn and early winter months. During the spring months, when all the glands of uncastrated males were actively secreting, a few glands from castrated males were still in the colloid condition, although the majority were actively secreting. No castrated animals were obtained after May 13.

3. Females (yearlings and lambs). The glands of mature females, aged from 8 to 11 months, were actively secreting during the period October to December. No glands from young females were obtained during the spring months. The glands of two females, aged 15-16 months, obtained in May, were in the colloid condition. From the beginning of May to August the glands from female lambs, aged from 3 to 7 months, contained large amounts of colloid like those of male lambs during the same period, but in addition many of the females were producing new secretion.

The glands of both sexes were heavier during the late spring and summer than during the autumn and early winter.

Influence of Age.

The glands of young mature sheep of both sexes showed a definite seasonal variation. The glands of immature lambs were only obtained during the summer months, and at this season the glands of both sexes contained large amounts of colloid, although the females were producing a certain amount of new secretion. In the glands of aged animals the colloid condition was the rule and the glands did not appear to respond to sexual or seasonal influences.

Influence of Locality.

The data were insufficient to allow of definite conclusion. The glands of two young males obtained from Yorkshire in October were exceptional in that they were actively secreting, whereas those of the Welsh males were in the colloid condition.

During the summer no difference was found between the condition of Irish spring lambs and Welsh spring lambs.

Influence of Sex.

The facts regarding the histological condition of the glands of normal males and females are summarized under 'Influence of Season'.

The glands of young mature males were always considerably heavier than those of young mature females.

Castration.

The facts regarding the influence of castration on the histological condition of the gland are also summarized under 'Influence of Season'.

During the autumn months the glands of castrated males were lighter than those of uncastrated males or females. During the spring months the glands of castrated males were much lighter than those of uncastrated males. Females were not weighed during this period.

Pregnancy.

Very few data were available. The glands of three pregnant females were heavier than that of one normal female obtained

on the same date and from the same district. All four animals were aged. The intervesicular tissue was more goitrous in the pregnant than in the non-pregnant animals.

REFERENCES.

1. Bamber, R. C. (1924).—"An apparent connexion between Braxy and Thyroid Activity", 'Nature', vol. 113, p. 161.
2. Blair Bell, W. (1920).—"The Sex Complex." London.
3. Fenger, F. (1914).—"Influence of castration and pregnancy on the Iodine and Phosphorus Metabolism of the Thyroid", 'Journ. Biol. Chem.'
4. Hunter and Simpson (1914).—"Influence of a diet of marine algae upon the Iodine-content of Sheeps' Thyroid", *ibid.*, pp. 119-22.
5. McCarrison, R. (1917).—"The Thyroid Gland in health and disease." London.
6. — (1925).—"Some problems of Thyroid disease", 'Brit. Med. Journ.', p. 1065.
7. Marine and Lenhart (1909).—"Colloid Glands (Goitres), their Etiology and significance", 'Bull. Johns Hopkins Hosp. Balt.', vol. xx, p. 131.
8. Martin, N. H. (1912).—"Brit. and Colonial Druggist", lxii, p. 99.
9. Mills, C. A. (1918).—"Effects of external temperature, Morphine, Quinine, &c., on Thyroid activity", 'Amer. Journ. Physiol.', 46, pp. 329-39.
10. Scidell, A., and Fenger, F. (1912-13).—"Seasonal variation in the Iodine-content of the Thyroid Gland", 'Journ. Biol. Chem.', xiii, p. 517.
11. Simpson, Sutherland (1913).—"Age as a factor in Thyroidectomy in Sheep", 'Quart. Journ. Exper. Physiol.'
12. Vincent, S. (1922).—"Internal Secretion and the Ductless Glands." London.
13. Vines, H. W. (1924).—"The Parathyroid Glands in relation to disease." London.

EXPLANATION OF PLATES 24 AND 25.

PLATE 24.

Fig. 1.—Part of transverse section of thyroid of 9-month-old uncastrated male sheep obtained from Ruthin on October 21. Gland in colloid condition with flat epithelium and thickened intervesicular tissue.

Fig. 2.—Part of T.S. of thyroid of 9-10-month-old female sheep obtained from Trefnant on November 5, showing gland in actively secreting condition with columnar epithelium.

Fig. 3.—Part of T.S. of gland from castrated male sheep (11 months old),

obtained from Abergele on December 9, showing colloid condition with low cuboidal epithelium.

Fig. 4.—Part of T.S. of gland from aged, non-pregnant female obtained from Wrexham on February 10, showing gland in colloid condition.

Fig. 5.—Part of T.S. of gland from aged pregnant female obtained on same date and from same district as female (fig. 4), showing colloid condition of gland with evidence of previous hypertrophy. Interventricular tissue more opaque than in fig. 4.

Fig. 6.—Part of T.S. of gland from yearling uncastrated male obtained from Corwen on April 22, showing actively secreting condition. Epithelium mainly columnar.

PLATE 25.

Fig. 7.—Part of transverse section of gland from castrated yearling obtained from Denbigh on April 29, showing actively secreting condition with columnar epithelium.

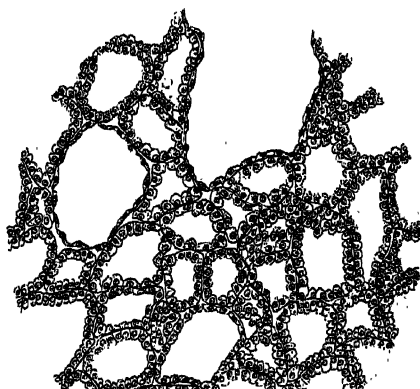
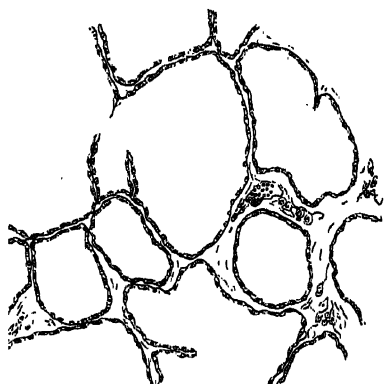
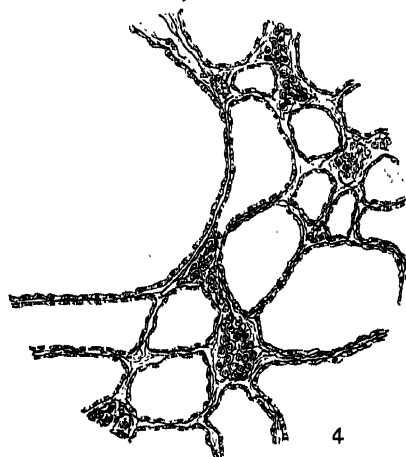
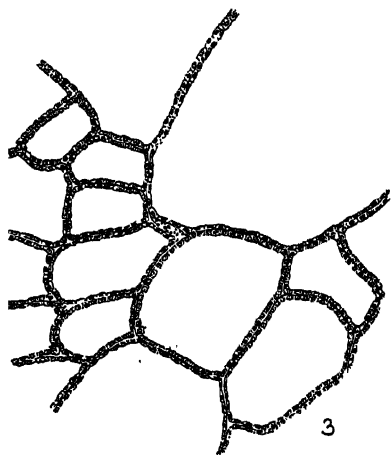
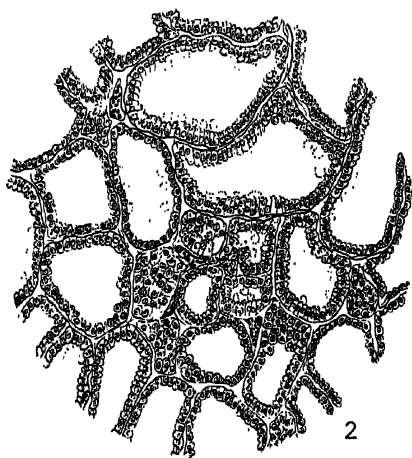
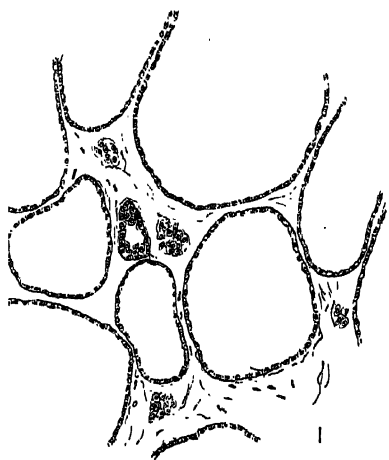
Fig. 8.—Part of T.S. of gland from 3-year-old castrated sheep obtained from Denbigh on April 29, showing gland in colloid condition with thickened interventricular tissue.

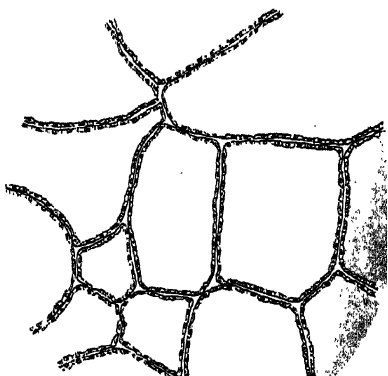
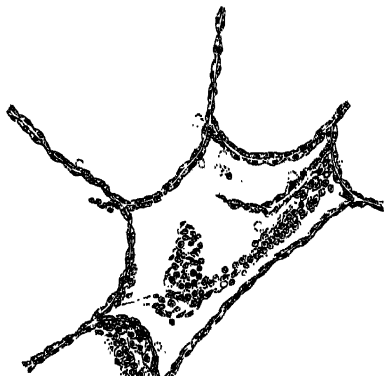
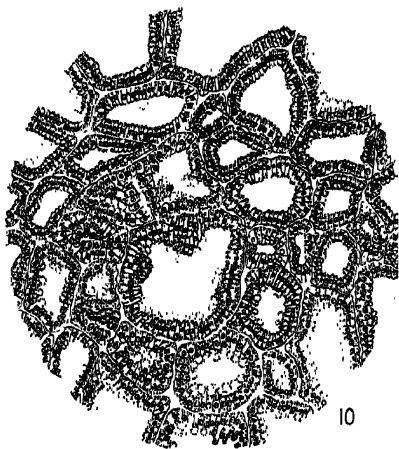
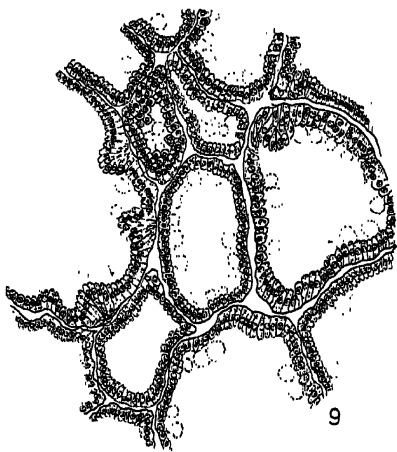
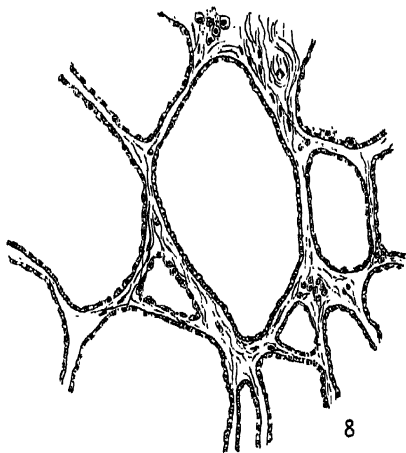
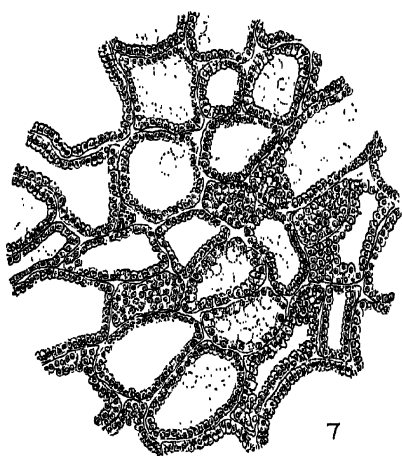
Fig. 9.—Part of T.S. of gland from castrated yearling obtained from Denbigh on May 13, showing very actively secreting condition accompanied by hypertrophy.

Fig. 10.—Part of T.S. of gland from uncastrated male yearling of same age as castrated male (fig. 9) obtained on same date and from same district. Gland in similar condition to fig. 9, but with less colloid.

Fig. 11.—Part of T.S. of gland from female yearling obtained on May 27 from Trefnant, showing colloid condition with evidence of previous hypertrophy.

Fig. 12.—Part of T.S. of gland from uncastrated male yearling obtained on same date and from same district as female (fig. 11), showing colloid condition.





The Green Bodies of the Intestinal Wall of certain Chaetopteridae.

By

C. Berkeley.

With Plate 26.

INTRODUCTION.

CLAPARÈDE (1) seems to have been the first to attribute the colour of the intestinal wall of *Chaetopterus variopedatus* Renier to the presence of small spherical green granules in the epithelial cells of the tissue. The distribution of the granules in the cells was subsequently described by Laffuie (2) and by Lankester (3). These three authors considered that they consisted of a pigmented cell secretion concerned in one way or another with digestion.

Brandes (4), on the other hand, expressed the view that the granules were not to be looked upon as a metabolic product of the worm, but were organisms of an algal nature analogous to those which had previously been described living symbiotically or parasitically in various animals by Brandt (5) and others. He based this view on the general appearance of the granules as figured by Lankester and on the similarity between the absorption spectrum of the green pigment chaetopterin extracted from them and that of chlorophyll. Enders (6), commenting on this difference of view, suggests that, if Brandes is correct, it should be possible to cultivate the green organism outside the worm as Famintzin (7) and Beijerinck (8) did in the case of *Zoochlorella* from *Hydra viridis*.

Since that time many instances of algal and animal associations have been recorded, but no conclusive evidence appears to have been brought forward in the case of *Chaetopterus*.

The common occurrence of three species of Chaetopteridae, each representing a different genus of the family, in the immediate vicinity of the Biological Station, Nanaimo, B.C., in each of which the intestinal tract is visible through the body-wall of the abdominal region as a well-defined deep green tract, suggested that some information on the nature of the pigmented bodies in the epithelial cells might be obtained by a comparative study.

The three species are: (1) *Mesochaetopterus taylori* Potts; (2) *Phyllochaetopterus prolifica* Potts; and (3) *Leptochaetopterus pottsi* Berkeley. They have all been recorded, and the first two described, by Potts (9); the third, only briefly mentioned by him and attributed to the genus *Telepsavus*, was described more fully by my wife (10). *Mesochaetopterus* and *Leptochaetopterus* inhabit tubes which run a considerable distance into the sand-banks in which they are found, the former frequently two feet or more in an almost vertical direction and then as far in a more horizontal one, the latter about half these distances. In neither case does the posterior end of the tube emerge to the surface as in that of *Chaetopterus variopedatus*. The animals are usually at least half the length of their tubes, and the abdominal region makes up by far the greatest part of their length. *Phyllochaetopterus* is found in tubes occurring in matted masses on gravelly bottoms at a depth of 15 to 20 fathoms, and is obtained easily in the dredge. Potts points out that it has the interesting faculty of increasing asexually, posterior portions of individuals splitting off and regenerating the anterior regions. He did not observe the sexual forms, but these are occasionally found.

Preliminary examination showed that the green coloration of the intestinal wall was due in all three cases to spherical granules very similar to those described by Lankester from *Chaetopterus variopedatus*. A pigment having the properties of chaetopterin could be extracted from each of them. It is easily soluble in strong hydrochloric acid (50 per cent. 'pure' HCl), in which it seems to be quite stable even on prolonged boiling, differing thus very essentially from chlorophyll. No

phaeococyanin, or other water-soluble pigment, could be extracted from the green tissue.

The tissue from each of the three species was examined by means of sections and the green bodies from teased-out material by hanging drops and dry films.

SECTIONS.

The green colour of the intestinal wall remains permanent in material fixed in sublimate and bichromate (Zenker's mixture) or picro-formol (Bouin). In transverse sections of the abdominal region of *Mesochaetopterus* or *Leptochaetopterus* thus fixed the intestinal wall is seen at low magnification to consist of a heavily pigmented, much convoluted, tissue. The intestine occupies a larger part of the abdominal cavity than in *Chaetopterus variopedatus*, and the lumen is much smaller in relation to the thickness of the wall (fig. 1, Pl. 26). Much the same condition exists in *Phyllochaetopterus*, but in this case the intestinal wall is relatively thinner and less convoluted and the lumen larger.

At higher magnification the intestinal wall is seen to be built up of the usual two layers of cells, the columnar tissue being similar to that figured by Lankester and others for *Chaetopterus variopedatus*. In sections taken anywhere except near the anal end the cells of this tissue are crowded with spherical green bodies varying in diameter from 1 to 8μ (fig. 2, Pl. 26). In *Mesochaetopterus* they are usually an olive shade, but some variation occurs in the depth of colour. In *Leptochaetopterus* it is commonly lighter and more blue-green. In both these species the green bodies are as a rule quite homogeneous and no structure can be brought out by staining. Quite rarely a few individuals have been seen in the sections showing a granular structure. A circular area rather more refractive than its surroundings, seen in some of the smaller and more lightly coloured green bodies, was at first taken for a nucleus, but it could not be differently stained. I shall refer to this later. In *Phyllochaetopterus* there is more variation in the colour of the green bodies than in either of the other species, some of them approxi-

ming to a blue colour, and a considerable number of them show distinct granular structure (fig. 3, Pl. 26). The granules are accentuated in sections stained with both nuclear and cytoplasmic stains. In addition to the more or less uniformly distributed granules a roughly circular area staining selectively with haematoxylin occurs in some of the green bodies and may be a nucleus.

In sections taken near the anal end of the annelids a different condition is found. Here the green bodies are uniformly smaller than in the more anterior region of the abdomen, averaging no more than 2μ in diameter and are not distributed throughout the epithelial cells. The majority are concentrated at the ends which border on the lumen, but a few are found scattered through the cells, usually lying immediately adjacent to the cell-wall (fig. 4, Pl. 26). The nearer the anal end the section is taken the fewer are the green bodies in the cells, until, finally, they are entirely absent from the last few segments.

In the course of his studies of the development of *Chaetopterus variopedatus*, Enders found that the green colour of the digestive tract could be observed in the larvae at an early stage, and that by the time they had attained a length of 2 mm. it had become quite marked, though less so than in fully transformed larvae. Free-swimming chaetopterid larvae of about this length have been taken over the beds in which *Mesochaetopterus* and *Leptochaetopterus* occur, and probably belong to one or other of these species. In these larvae the colour of the digestive tract was quite apparent and sections showed that it was due to green bodies in the epithelial cells precisely similar to those found in the adults. The conditions of size and of density of distribution in the cells resembled those found in sections taken near the posterior end of the adult animals.

In material fixed in acid alcoholic fixatives the pigment is entirely removed from the green bodies. A fixative consisting of 95 per cent. methyl alcohol (100 parts), water (20 parts), formalin (5 parts), and glacial acetic acid (5 parts) was used for this purpose. Pieces of abdomen of *Mesochaetopterus* left in this fixative until decolorization was complete (some

months) were stained in bulk in borax-carmin. Sections showed stained, but shrunken and deformed, residues of the formerly green bodies in the epithelial cells. More satisfactory results were obtained by fixing for a short period in the alcoholic fixative and completing the decolorization, in the sections, with alcohol containing hydrochloric acid (10 drops conc. HCl to 100 cc. 95 per cent. alcohol). In sections so treated the green bodies were much less deformed. They could be stained with any of the ordinary cytoplasmic stains. Lankester mentions a 'colourless stroma' which remains after the green bodies in *Chaetopterus variopedatus* have had their pigment removed, and this corresponds without doubt with the decolorized residuum in my sections. The fact that such a stainable residuum remained after decolorization seemed to indicate that the green bodies had something of the nature of a bounding membrane or cell-wall, and to be opposed to the view that they consisted of a cell secretion.

Sections of material fixed in osmic acid fixatives showed that the green bodies contain a quantity of oil. They are stained intensely black, whilst the remainder of the contents of the epithelial cells is uncoloured.

HANGING DROPS.

The green bodies can be easily separated from the tissue containing them by teasing it out in water, filtering through fine bolting silk, and fractionating the filtrate by means of a centrifuge. A great deal of oil is present in this filtrate, but it remains in suspension on centrifuging, and after a few washings a residue is obtained consisting of little but the green bodies and bacteria.

Examination of such material, freshly isolated from *Phyllochaetopterus*, in a hanging drop showed immediately that the green bodies were discrete organisms. A large number of them were seen to be in active independent movement, and flagella could usually be detected on the motile individuals. A trace of basic fuchsin added to the drop brought these out quite clearly. Two flagella occur on each individual, and they are of

approximately equal length, at least twice as long as the diameter of the organism bearing them (fig. 5, Pl. 26). They are carried on a minute protuberance which appears as a bright spot when the organism is swimming in such a position that it is seen through the green body. This is without doubt the refractive area previously referred to as having been seen in sections of *Mesochaetopterus* and *Leptochaetopterus*, and taken for a nucleus. Both homogeneous and granular individuals, of all sizes except the largest, showed motility.

The organisms in a fresh suspension prepared from the anterior part of the intestine of *Mesochaetopterus* or *Leptochaetopterus* showed no motility, but after standing in a cool room for twenty-four hours, many motile individuals were present and flagella could be detected. After forty hours the majority of the green cells were in active movement. In the case of *Leptochaetopterus* some granular cells, similar to those seen in *Phyllochaetopterus*, were present, and these showed motility as freely as the homogeneous forms. In the material from both *Mesochaetopterus* and *Leptochaetopterus* almost colourless cells of the same size and shape as the green bodies occurred and were in many cases motile and flagellated. The flagella are the same length, relative to the individual organism bearing them, as in *Phyllochaetopterus*, and are similarly carried. The organisms from the three species of annelid cannot in fact be differentiated except to some extent by colour and by the greater frequency of granular forms in the case of *Phyllochaetopterus*.

If the green bodies are isolated from segments near the anal end of *Mesochaetopterus* or *Phyllochaetopterus*, actively motile individuals are found amongst them immediately. They are of smaller size in this case, averaging about 2μ in diameter, and no structure can be made out. A tendency to greater frequency of motility, when freshly extracted, amongst the green bodies taken from near the tail than amongst those taken further anteriorly was subsequently found to exist in *Phyllochaetopterus* also, particularly in the longer sexual individuals which are found occasionally. In the usual asexual

forms the abdominal region is short, and since they are constantly being budded off the anal end of the parent stock, the anterior abdominal segments are of recent growth compared with the corresponding segments in *Mesochaetopterus* and *Leptochaetopterus*. This greater approximation in age of anterior and posterior segments in asexual *Phyllochaetopterus* is accompanied by a corresponding decrease in the difference between the two regions in respect of the frequency of occurrence of motile individuals.

Dividing cells were seen in all the hanging drops examined. The two cells resulting from division separate and become spherical before division takes place again, so that no chains or definite colonial groupings are formed. Both homogeneous and granular individuals of all sizes, except the smallest, are found dividing, and occasionally a pair is seen made up of one of each kind (fig. 6, Pl. 26). No contractile vacuole, nucleus, or pyrenoid could be made out in the living organisms, nor, their colour being uniformly distributed, any definite chromatophore.

FILMS.

Attempts were made to obtain further information on the structure of the green cells by making films from the suspensions obtained, as described in the preceding section, from each of the three species and submitting them to the action of reagents. Drops of the material were allowed to dry on slides smeared with glycerine-egg albumen and fixed for varying periods in the alcoholic fixative previously mentioned. The organisms were thereby more or less decolorized. Very little deformation of the cells seemed to result from this treatment.

Chlor-zinc-iodine failed to indicate the presence of a cellulose cell-wall. The chaetopterin was quickly dissolved out of the cells, if they were not already completely decolorized, and the cells soon disintegrated. No blue or violet reaction could be detected in any completely decolorized cells.

Iodine, either in alcoholic or sodium iodide solution, gave no starch reaction. In the case of *Phyllochaetopterus* and *Leptochaetopterus* inclusions staining a reddish brown

were seen in a few cells which may indicate the occasional presence of glycogen as a storage product.

The decolorized cells could be recoloured with cytoplasmic stains. The structure in the granular individuals could best be brought out by staining with methylene blue, haematoxylin (Ehrlich's) or iodine. In some of the larger cells fine strands could be seen connecting the granules recalling the condition found in some of the unicellular Cyanophyceae (fig. 7, Pl. 26).

Several individuals differing in appearance from the usual type were found in films prepared from *Phyllochaetopterus* (fig. 8 *a* and *b*, Pl. 26). In those shown at (*a*) the pigmented cell contents have concentrated into centres in a more or less regular manner. In those shown at (*b*) the formation of reproductive bodies seems to be involved. The cells contain smaller discrete bodies, but it has not been possible to count their number. Their expulsion from the parent cell has not been observed, but empty cells of large individuals are found in the films alongside a number of very small ones suggesting very strongly that the former has given rise to the latter.

Neither granular cells nor this apparent spore formation has been seen in normal *Mesochaetopterus*, but when a portion of the abdomen of an individual of this species was kept in water in a closed vessel for some weeks, until the tissue had entirely decomposed, the green organisms not only remained intact, but were found, on examination in hanging drops and films, to be in an active state of division, and both granular forms and individuals showing suspected spore formation were present.

In cells obtained from *Phyllochaetopterus* and *Leptochaetopterus* a more or less central, roughly circular body, staining well by prolonged treatment with Ehrlich's haematoxylin (diluted 1 : 5), is frequently present, which possibly may be a nucleus.

Sudan III applied directly to films made from each of the three species confirmed the evidence obtained from the sections of material fixed with osmic acid fixatives that the green cells contain a considerable quantity of oil. As a rule the oily material

is extruded from the cells on drying and is shown in the films stained with Sudan III as a red field surrounding each group of green cells.

Dividing cells, as described in the previous section, were found in all the films.

DISCUSSION.

The colour of the intestinal wall of the three species of Chaetopteridae dealt with in this paper is, then, due to an infection of a green unicellular organism. Considering the very similar character of the green bodies in *Chaetopterus variopedatus* and of the pigment extracted from them, the explanation is almost certainly the same in this case also. Infection has never been found absent in either of the three cases investigated, and it takes place at a very early stage of the development of the annelids. This is not remarkable since the infective organism is always present in the contents of the gut and in the faecal masses, and the sexual products are carried out of the tubes by the same current of water as carries away the excreta. In a large majority of the cases in which the eggs of *Mesochaetopterus* have been examined they have been found to carry the green infective organism externally.

The organism remains normally in the palmella stage and increases only by fission; it is found always in this condition in the older intestinal tissue. The flagellated form is found only in the recently generated segments and is clearly the agency by which the infection is carried into new areas. It can be assumed by individuals of various sizes under conditions favourable to swarming, as for instance in a suspension of the material teased out of the older tissue after standing some time, but, judging by the size of the organisms found in the larvae and in the recently budded segments, infection is normally brought about by the smaller individuals.

There is no evidence on which a final opinion can be based as to whether the green organism is to be regarded as parasite or symbiont, and it is unlikely that the point can be decided with certainty unless the annelids can be bred under controlled

conditions, which hitherto has proved impossible. The epithelial cells of the gut are no doubt concerned in the disposal of organic waste material resulting from the digestive processes of the annelid. Probably, therefore, they afford an ideal environment for the development of the green organism, but there is no evidence that the annelid benefits in any way by the association. In the cases of *Convoluta roscoffensis* and *Convoluta paradoxa*, Keeble (11) has shown that, in addition to supplying carbohydrate and fat resulting from photosynthesis, the algae associated with these animals serve in lieu of excretory apparatus by absorbing their nitrogenous waste materials and are thus indispensable to their hosts. Photosynthesis is precluded in the case of the chaetopterids owing to the conditions of total darkness in which the green organisms live, but it seems not unlikely that they play a similar part to the algae in *Convoluta* by assisting in the disposal of the animals' waste products, and since they are always rich in oil, it is probable that the large quantity of that substance found in the intestinal cells results from their synthetic activity and serves a useful purpose in the annelid's metabolic processes. In this case the association must be regarded as symbiotic.

On the assumption that the green organism absorbs the waste products of the worm, attempts have been made to grow it in liquid and on solid media containing uric acid and allied substances as nitrogenous nutrients, but although a large number of media have been tried under a variety of conditions, no success has yet been attained.

So far as can be judged without having the organisms isolated in pure culture they are very similar in all the three chaetopterids examined, though the slight morphological differences which have been mentioned may indicate varietal adaptations to each species. Without such cultures it may not be possible finally to determine the systematic position of the organism. It is evident that it is not an alga, as suggested by Brandes, if that term is used in its restricted sense to mean a definitely vegetable organism adapted to photosynthesis, as in the cases of *Zoochlorella* in its various associations, the *Chlamydomonas*

described by Keeble, and other similar instances, since it has neither cellulose, cell-wall, chromatophore, nor pyrenoid, and appears to contain no algal pigment. It comes within the term if it is defined in its widest sense to comprise all the coloured Flagellata. According to Pascher's (12) classification of the pigmented flagellates, it must be included in the Chrysomonadae, though it fails in some respects to be a typical member of the group. Its characteristic of living normally in the palmella stage would then place it amongst the Chrysocapsinae, and it would come most nearly within the genus *Chrysocapsa*. The name *Chrysocapsa chaetopteri* is therefore tentatively suggested for the species.

I wish to record my thanks to the Biological Board of Canada, and to Dr. Clemens, Director of the Pacific Biological Station, for the facilities placed at my disposal which have enabled me to carry out this investigation.

SUMMARY.

1. The green bodies in the intestinal epithelial cells of three species of Chaetopteridae, each representing a different genus, have been examined.

2. They are very similar in the three cases and resemble those of *Chaetopterus variopedatus*.

3. They are frequently motile and flagellated when separated from the intestinal tissue.

4. They represent an infection of an organism which spreads in a flagellated condition.

5. The organism is a flagellate probably belonging to the family Chrysocapsinae.

6. The name *Chrysocapsa chaetopteri* is proposed for it.

LITERATURE CITED.

1. Claparède, E.—'Struct. Annélides Sédentaires', 1873.
2. Laffuie, J. J.—"Étude monograph. du *Chaetopterus* var. *Chaetopterus variopedatus*", 'Arch. de Zool. Exp. et Gén.', Series 2, vol. 8, 1890.
3. Lankester, R.—"On the Green Pigment of the Intestinal Wall of the Annelid *Chaetopterus*", 'Quart. Journ. Micr. Sci.', New Series, vol. 40, 1897.

4. Brandes, G.—“Die Ursache der Grünfärbung des Darmes von *Chaetopterus*”, ‘Zeit. f. Naturw.’, vol. 70, 1898.
5. Brandt, C.—“Über die Morphologische und Physiologische Bedeutung des Chlorophylls bei Thieren”, ‘Archiv f. Anat. und Physiol., Abth. f. Physiologie’, 1882.
6. Enders, E. E.—“A Study of the Life-history and Habits of *Chaetopterus variopedatus*,” Renier et Claparède, ‘Journ. of Morpholog.’, vol. 20, no. 3, 1909.
7. Famintzin, A.—“Beiträge zur Symbiose von Algen und Thieren”, ‘Mém. Acad. Imp. Sci.’, vol. 38, 1890.
8. Beijerinck, M. W.—“Culturversuche mit Chlorzellen, Lichtergonidien und anderen niederen Algen”, ‘Bot. Zeit.’, vol. 48, 1890.
9. Potts, F. A.—“Polychaeta from the north-east Pacific. The Chaetopteridae. With an Account of the Phenomenon of Asexual Reproduction in *Phyllochaetopterus* and the Description of two new Species of Chaetopteridae from the Atlantic”, ‘Proc. Zool. Soc. London’, 1914.
10. Berkeley, E.—“A New Genus of Chaetopteridae from the north-east Pacific, with some remarks on Allied Genera”, *ibid.*, 1927.
11. Keeble, F.—‘Plant-Animals. A Study in Symbiosis.’ Camb. Univ. Press, 1912.
12. Pascher, A.—‘Die Süßwasser-Flora Deutschlands, Österreichs und der Schweiz’, Heft 2, Jean, 1913.

EXPLANATION OF PLATE 26.

Fig. 1.—Transverse section of median region of intestine of *Mesochaetopterus taylori*.

Fig. 2.—A few cells of above highly magnified.

Fig. 3.—A few cells from a transverse section of the median region of the intestine of *Phyllochaetopterus prolifica*.

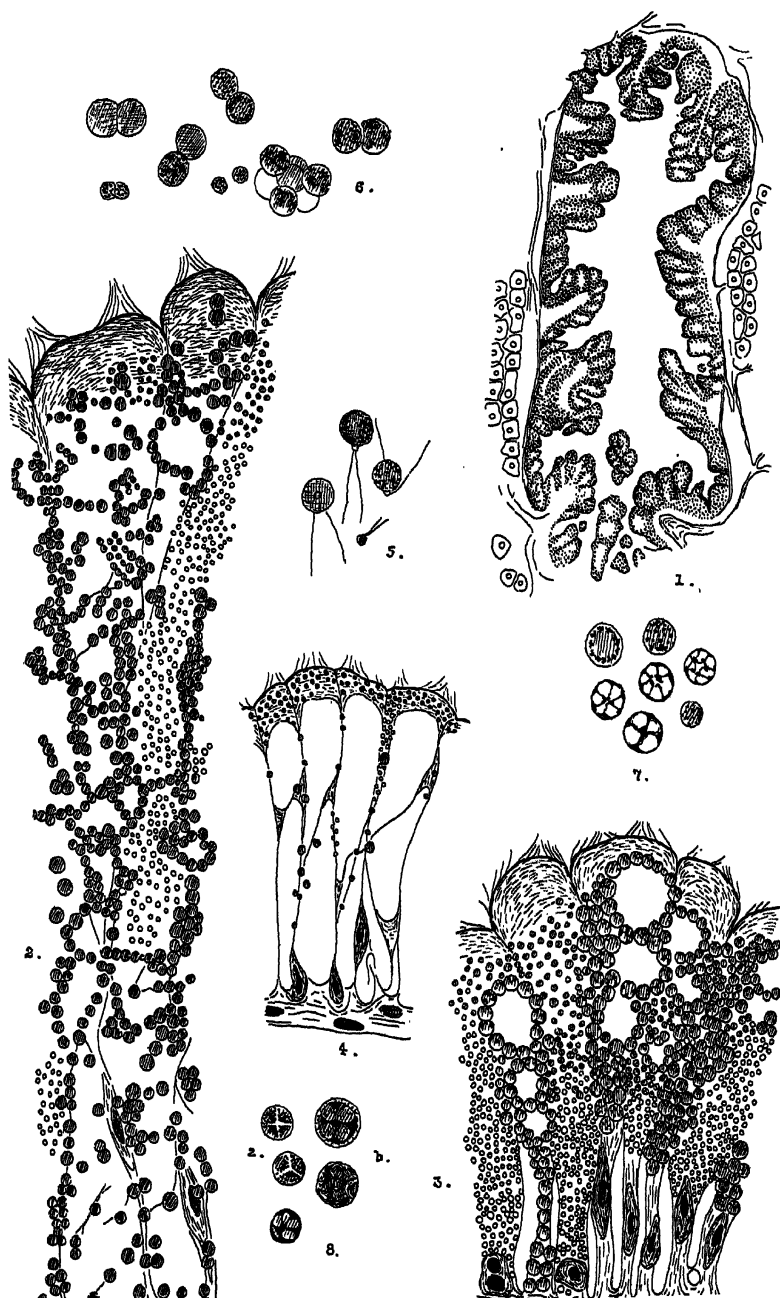
Fig. 4.—Cells from transverse section of intestine taken near the anal end of *Mesochaetopterus taylori*.

Fig. 5.—Motile green organisms from *Phyllochaetopterus prolifica*.

Fig. 6.—Stages in fission of green organisms from *Phyllochaetopterus prolifica*.

Fig. 7.—Granular green organisms from *Phyllochaetopterus prolifica*.

Fig. 8.—Specialized green organisms from *Phyllochaetopterus prolifica*.



Studies on the shape of the Golgi Apparatus.
II. Observations on the fresh Egg of the
Indian Earthworm, *Pheretima posthuma*.

By

Vishwa Nath, M.Sc., Ph.D. (Cantab.).

Department of Zoology, Government College, University of the Panjab,
Lahore.

With 17 Text-figures.

INTRODUCTION.

IN the first paper (1929) of this series on the egg-follicle of *Culex fatigans* I have shown that the Golgi elements in the oocytes, in the nurse-cells, and in the follicular epithelial cells are in the form of vesicles, each vesicle showing an osmiophilic or argentophilic rim and an osmiophobic or argentophobic central area. The contents of the Golgi vesicles of the nurse-cells and of the follicular epithelial cells remain non-fatty throughout the growth period of the egg-follicle ; while those of the majority of the oocyte vesicles become fatty, although they do not swell up. If a fresh egg is ruptured and its contents studied under the microscope the Golgi vesicles are seen performing a very interesting dancing movement while the proteid yolk bodies are stationary. The presence of fat inside the interior of the Golgi vesicles raises their refractive index and enables one to study them in fresh eggs with the utmost ease. Vesicular Golgi elements have also been demonstrated by the writer (1928) in the eggs of a spider, by Nath and Husain (1928) in the eggs of a Scolopendrid, by Nath and Mehta (1929) in the eggs of *Luciola*, and by Nath and Piare Mohan (1929) in the eggs of the cockroach. It is important to note that in all the above cases the Golgi elements were traced from the youngest to the most highly advanced oocytes, and in some cases (*Culex* and *Luciola*) vesicular Golgi elements were observed

even in the undifferentiated primordial germ-cells. In the case of the cockroach such elements were found even in the terminal thread of the ovary. The above conclusions were based not only on the study of fixed preparations, which by themselves are entirely worthless at least for the study of the morphology of the Golgi apparatus, but also on the study of fresh cover-slip preparations treated with vital dyes or with 2 per cent. osmic acid for a very short time.

The Golgi element in the eggs then is a vesicle consisting of two fundamentally different substances, namely, the osmophilic peripheral rim and the central clear osmiophobic substance. The writer has always been puzzled by the 'crescents' or the 'dictyosomes', forms under which the Golgi elements have been constantly described in the male germ-cells by such competent writers as Gatenby and Bowen. But a complete and final solution of this puzzle has unexpectedly come in the form of an excellent publication on the Golgi apparatus and vacuolar system of *Cavia*, *Helix*, and *Abraxas* by Gatenby (1929). This writer has shown that even in the male germ-cells there is a number of neutral red-staining vacuoles lying near, but not attached to, the Golgi dictyosomes. In some cases (e.g. *Helix*) the vacuoles are in the archoplasmic area at the periphery of which the Golgi dictyosomes lie, while in others (e.g. *Cavia*, *Saccocirrus*, and *Abraxas*) they are extra-archoplasmic. These vacuoles are distinct from the Golgi dictyosomes not only in the 'resting' cells but also during the meiotic division when they are sorted out roughly into two equal parts. It is very important to note that, according to Gatenby, these vacuoles are not consistently argentophilic, whereas the dictyosomes are. Gatenby also gives a useful review of the recent work of Hirschler, Monné, and Voinov on the male germ-cells of lizard and grasshopper, *Cerithium* and *Helix*, and *Notonecta* respectively. These authors, as quoted by Gatenby, have also shown a vacuolar system lying near, but distinct from, the Golgi dictyosomes.

Parat's views on the homologies of the cell-constituents of the *Helix* spermatid, which are well known, are diametrically

opposite to those of Gatenby, Hirschler, &c. Parat considers the archoplasmic vacuoles as the Golgi apparatus and the cortical dictyosomes as the modified mitochondria or the 'lepidosomes'. The evidence brought forward by Gatenby and others, however, seems to be overwhelming, and one cannot have any hesitation in accepting their views.

The paper of Gatenby cited above lends strong support to my views on the shape and function of the Golgi elements in oogenesis. I have consistently described the Golgi elements in eggs as vacuoles or vesicles, each vesicle having an osmiophilic or argentophilic rim. According to Gatenby, there exists in the animal cell a vacuole or a system of vacuoles primitively associated with, and probably produced by, the argentophil cortex of the Golgi apparatus. Further, in the eggs the vacuole is 'closely related to the chromophil substance of the Golgi apparatus'. 'The work of Miss A. G. Hill in this laboratory has convinced the writer that in such examples of oogenesis as that of *Daphnia*, the Golgi element is a cortex on the vacuole, and the division of the element brings about a division of the associated vacuole.' In showing that the mysterious 'idiosome' or the 'sphere substance' which in many cases appears as the chromophobe part of the Golgi element is nothing but a collapsed vacuole, Gatenby has made a first-class contribution to cytology.

It appears, therefore, that the essential part of a Golgi element is the peculiarly osmiophilic or argentophilic material which in the somatic cells occurs in the form of granules, rods, or a reticulum, while in the plant-cells it is found, as claimed by Bowen (1928) and by Patten and collaborators (1928), in the form of platelets which impregnate with osmic acid like the typical Golgi apparatus. Bowen has insisted that the plant vacuole is not osmiophilic, that is to say, it is a mere vacuole without the osmiophilic rim; and since Gatenby, Hirschler, &c., have shown a vacuolar system distinct from the true Golgi apparatus in the male germ-cells, one is driven to the conclusion that the theory of Guilliermond and Parat homologizing the plant vacuole with the Golgi apparatus cannot be any longer upheld.

If the plant vacuoles and the vacuoles of the male germ-cells are mere vacuoles and not the Golgi apparatus, it perhaps becomes doubtful if the neutral red-staining vacuoles which Parat and his collaborators have been describing in eggs are surrounded by a truly osmiophilic Golgi material. At any rate Nath and collaborators find that in the eggs of the spider (Nath, 1928), *Culex* (Nath, 1929), *Pheretima*, medicinal leech (Nath, unpublished), Scolopendrid (Nath and Husain, 1928), *Luciola* (Nath and Mehta, 1929), and the cockroach (Nath and Piare Mohan, 1929), the Golgi vesicles are not stainable, at least brilliantly as claimed by Parat, with neutral red even in the early stages of oogenesis when in some of the above cases (spider, Scolopendrid, *Culex*, and the cockroach) their contents are non-fatty.

PREVIOUS WORK ON THE EARTHWORM OVARY.

It is essential to discuss the remarkable papers of Foot (1894, 1896, and 1898), and of Foot and Strobell (1898, 1900, and 1901), on the cocoons and eggs of *Allolobophora faetida*. I consider this work of a very high order because it is based on an extensive study of fresh cover-slip preparations, and the authors have taken pains to publish a large number of excellent photographs. In those days very little was known about the mitochondria, and the Golgi apparatus was entirely unknown by zoologists. In spite of this the authors have given a very faithful account of oogenesis in this earthworm.

These authors (1901) described two types of granules in the egg of *Allolobophora*, namely, 'deutoplasmic' or 'osmiophile' granules and smaller 'archoplasmic' granules which arise from the disintegration of the 'yolk-nucleus' (Calkins, 1895). The osmiophile granules are present long before the disintegration of the yolk-nucleus, and they can be demonstrated during every stage of development of the egg. They are found in nearly all the cells of the ovary, from the small cells near the proximal end, which show the first indication of a yolk-nucleus, to the large oocytes at the distal end (Foot and Strobell, 1901, p. 518). These granules can be demonstrated during all

stages of the growth of the egg, the maturation, fertilization, and cleavage (p. 519). The yolk-nucleus granules fail to blacken with osmic acid, even after many hours' immersion in a 1 per cent. solution, whereas an immersion of fifteen or even five minutes is sufficient to blacken the osmiophile granules. The yolk-nucleus invariably stains intensely with haematoxylin, while the osmiophile granules very rarely react to this stain (p. 524). The osmiophile granules also show conspicuously in the living egg, and agree both in size and position with those seen in fixed material. They appear in the substance between the cells and in the small cells (oogonia?) near the proximal end of the ovary (p. 531). In the oogonia and in the oocytes of all stages the yolk-nucleus is morphologically an accumulation of granules. When the granules are scattered through the cytoplasm they are very difficult to demonstrate, but when they are aggregated into more or less definite masses they can be readily differentiated (p. 522).

It is convenient to anticipate at this stage that the 'osmiophile' granules and the 'archoplasmic' or 'yolk-nucleus' granules are the Golgi elements and the mitochondria respectively.

Harvey (1925), working on the egg of *Lumbricus terrestris*, however, arrived at the following conclusions:

1. The yolk-nucleus is merely a mass of mitochondria.
2. The mitochondria arise as a cap of threads over the nucleus.
3. The mitochondria are not clearly defined in the very young oogonia.
4. The Golgi apparatus consists of numbers of Golgi elements lying separate in the cytoplasm. There is never any attempt at concentration of these elements round one central mass.
5. The Golgi elements are probably little platelets or spheroids somewhat resembling blood-corpuscles in shape. They are not rods. As fixed by Da Fano technique, each element is a little plate with a very lightly impregnating centre and a very heavily impregnating rim.

6. The Golgi elements may probably arise from the cytoplasm.
7. Yolk is present, and probably arises from the cytoplasm.
8. No direct metamorphosis of either mitochondria, Golgi apparatus, or nucleolus into yolk was observed.

Unfortunately these conclusions were based entirely on the study of fixed preparations because 'the fact that the egg of *Lumbricus* is full of highly refractive granules and globules of yolk, fat, &c., which obscure any signs of the fine thread-like mitochondria, makes the study of the inclusions in the living cell impossible'.

Later, Gatenby and the writer (1926) published a paper on the same subject, but they disagreed with all the statements of Harvey except those contained in paragraphs 1 and 8. Their results were based not only on the study of fixed preparations, but, which is very important, on the study of fresh cover-slip preparations made by Gatenby alone, as the writer was compelled to leave Dublin for his home in June 1925. On his return to India he took the earliest opportunity of making much more extensive *intra vitam* and other observations on the egg of the common Indian earthworm. Indeed he has been demonstrating the mitochondria and the Golgi elements to his Honours School classes with the utmost ease, not only in the eggs of the earthworm but also in those of the medicinal leech. The cytoplasm of both these eggs is not encumbered with yolk, the only inclusions present being the Golgi elements and the mitochondria. For this and other reasons to be mentioned subsequently these two types of inclusions stand out prominently, making these objects truly classical for observations on living material.

The conclusions arrived at by the writer with regard to the oogenesis of *Pheretima* were substantially similar to those arrived at by him and Gatenby in the case of *Lumbricus*. In spite of the fact that he discovered important additional facts in support of the previous conclusions on *Lumbricus* (1926) and also in support of his views, which he holds very strongly, on the shape and function of the Golgi elements in eggs in general, he preferred not to go to press, hoping that the differences would automatically disappear when it would be

possible for Harvey to observe living eggs. That hope, however, has been only partially fulfilled as the following quotation will show (Harvey, 1929): 'The author would like to acknowledge here the correctness of Professor J. Brontë Gatenby's statement that the egg of *Lumbricus* can be easily studied in the living condition. He can only attribute his former failure with it to lack of experience in this particular field. Further observation has demonstrated to him that the Golgi bodies can be easily seen in these eggs, living and unstained. With Gatenby's further statements about this egg, however, he still remains in disagreement, and is strengthened in his own opinions by the re-examination of numerous samples. The author holds that the mitochondria are thread-like, not granular, that there is a certain amount of material, yolk for want of a better word, in the eggs, and he is not convinced that only one Golgi body is present in the oogonia and youngest oocytes.'

The above lines appear as a footnote in a paper on the oogenesis of the crab. The writer would have greatly welcomed a statement from Harvey with regard to the shape of the Golgi elements in the eggs of the earthworm which he now claims to have studied in the living condition, because the morphology of the Golgi element is of fundamental importance, particularly in eggs, and is inextricably bound up with their function.

OBSERVATIONS.

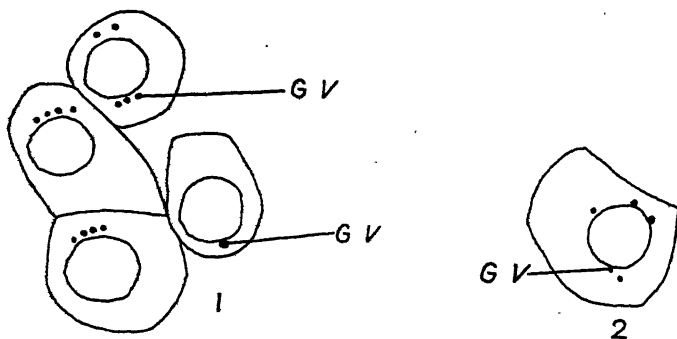
Fresh preparations.

It has already been mentioned that the earthworm ovary may be considered a very favourable object for the study of the mitochondria and the Golgi elements in the living condition without the help of any vital dyes. This is true, strange as it would appear, especially in the case of the Golgi elements which stand out much more prominently than the mitochondria in the freshly extirpated ovary. If the ovary is mounted flat on the slide in a drop of normal saline so that the oocytes are not allowed to cover each other, one can see the oocytes arranged in filaments radiating from the septal insertion of the ovary. The oogonia are proximal, that is to say, they lie near the septal

insertion and the oocytes lie more distally, the most highly developed oocyte lying at the free end of the filament. On account of this peculiarly favourable arrangement the study of different stages in one specimen only is greatly facilitated.

In text-fig. 1 is shown a group of four oogonia in which there is not a trace of any granules which can be assigned to the category of mitochondria, the cytoplasm having a hyaline glass-like appearance. The Golgi elements, however, stand out very

TEXT-FIGS. 1 and 2.



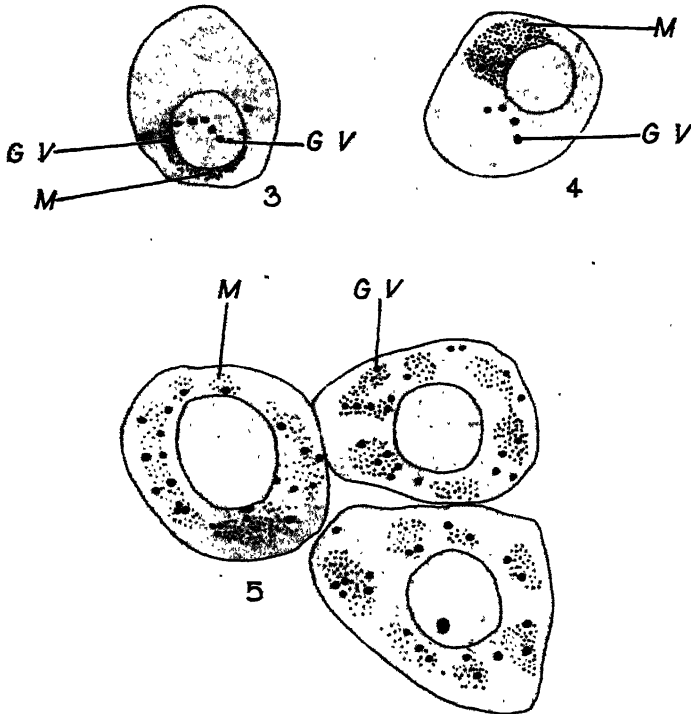
EXPLANATION OF LETTERING.

G.V.=Golgi vesicles. M.=Mitochondria. N.=Nucleolus.

Further explanation of figures will be found in the text. $\times 560$.

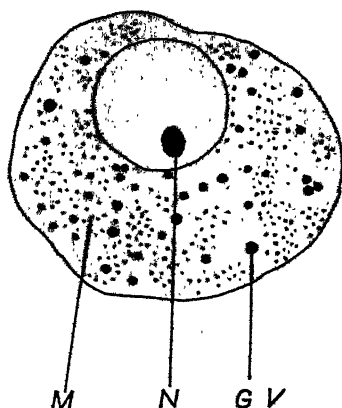
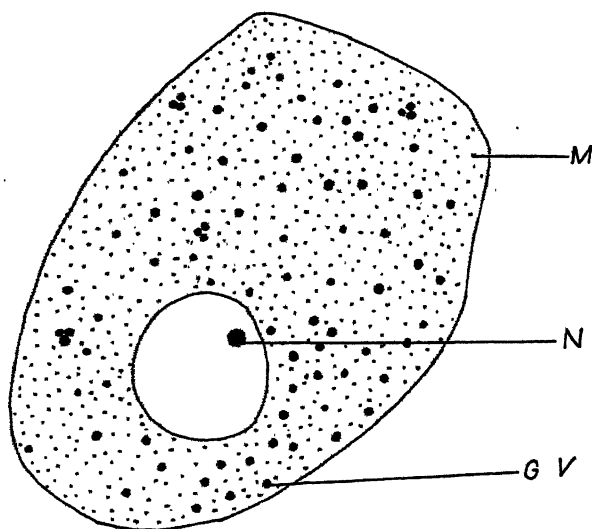
prominently as highly refractile spherules of a dark-greyish colour. In one oogonium in Text-fig. 1 there is a single Golgi element, while in others there are four or five such elements. I have frequently seen oogonia with one, two (Text-fig. 9a), four, or more Golgi spherules; but I have never observed three such spherules in an oogonium, strange as it may appear, although my studies have been spread over many years and numerous specimens. With the growth of the oogonium the Golgi spherules spread out in the cytoplasm (Text-figs. 2, 3, 4, 5, and 6) till in an advanced oocyte (Text-fig. 6a) they are distributed more or less uniformly throughout the egg. In the oocytes the Golgi elements perform a dancing movement which is very interesting to watch.

The mitochondria appear for the first time either as a thick mass (Text-figs. 4 and 9*b*) or as a horseshoe-shaped structure closely fitting the nuclear membrane (Text-figs. 8, 9*c* and *d*). Very soon the mitochondrial cap breaks away from the nuclear mem-



TEXT-FIGS. 3—5. $\times 560$.

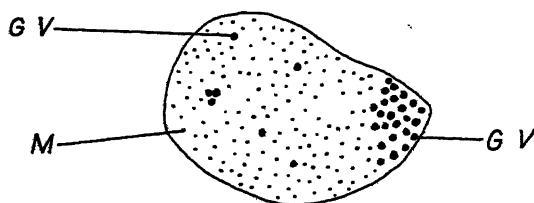
brane and is quickly resolved into aggregations of mitochondrial granules (Text-fig. 5). The mitochondrial aggregations now become loosened (Text-fig. 6) till in advanced oocytes the granules are distributed more or less uniformly throughout the cytoplasm (Text-fig. 6*a*). The mitochondria, even in the earliest cap, are in the form of very small, whitish granules with a refractive index much lower than that of the Golgi elements. They perform a very rapid dancing movement closely simulating the similar

TEXT-FIG. 6. $\times 280$.TEXT-FIG. 6a. $\times 560$.

movement of bacteria. The very much smaller size of the mitochondrial granules, their lower refractive index, their peculiar distribution in the egg, and their whitish appearance as compared with the dark-greyish colour of the Golgi spherules, are factors

which preclude any possibility of confusion between these two kinds of cytoplasmic inclusions.

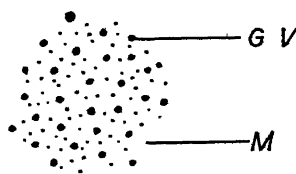
The nuclear contents consist of faint and fine fibres with a prominent semi-solid and refractile nucleolus. Near the septal insertion of the ovary some oogonial nuclei can be seen



TEXT-FIG. 7. $\times 560$.

in the prophase with much thicker and more conspicuous fibres inside them.

When some time has elapsed the egg begins to disintegrate

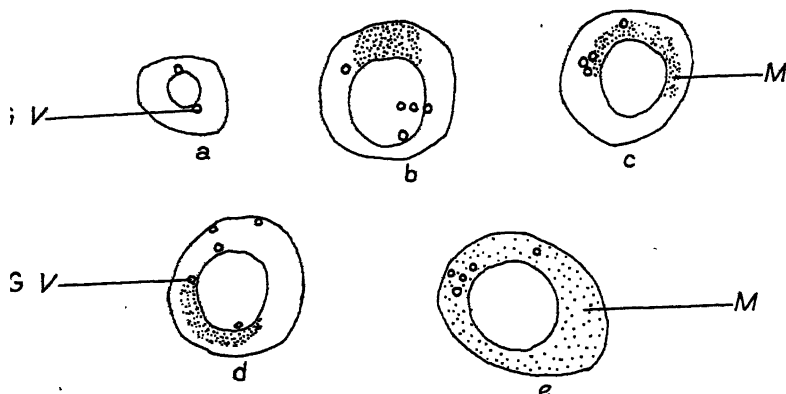


TEXT-FIG. 8. $\times 560$.

under the eye of the observer, and it breaks down into fragments which seem to be surrounded by a thin limiting membrane. In Text-fig. 7 such a fragment has been shown. Most of the Golgi elements are crowded in one corner and are perfectly stationary, but the mitochondrial granules which occupy the major portion of the fragment, with a few Golgi elements lying amongst them, perform a very rapid dancing movement which contrasts prominently with the stillness of the crowded Golgi elements in the corner.

In other cases at the time of the death of the egg the egg-membrane ruptures at one or several places and its contents

flow out in the form of a rapid stream containing the mitochondrial granules and the Golgi elements (Text-fig. 8). They remain unaltered for a long time after the death of the egg, showing that they have a firm consistency and are not so susceptible to injury as is generally supposed. The Golgi vesicles of the *Culex* egg also remain unaltered for a long time after the death of the egg.



TEXT-FIG. 9. $\times 560$.

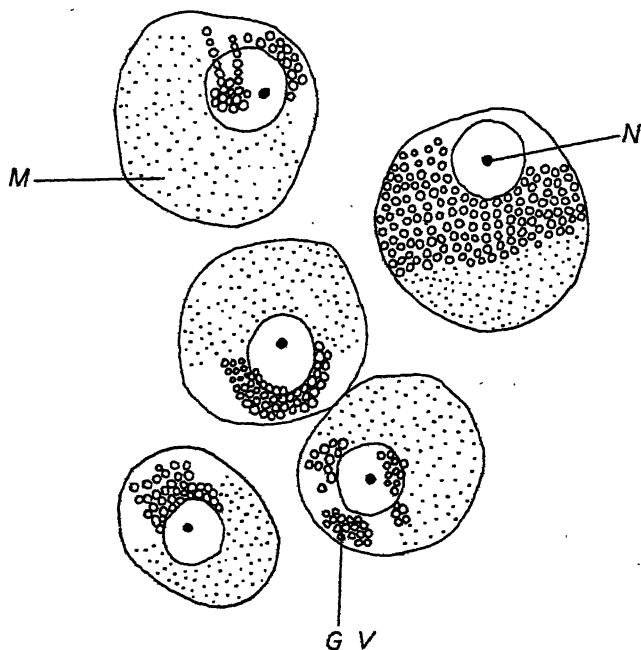
Vital dyes, namely, neutral red and janus green B, have been extensively used in the form of very thin watery solutions, but they do not in any way improve the appearance of the egg, if indeed an improvement were desired. The Golgi spherules do not stain at all with either of these dyes, but the mitochondria do take up very slightly a blue colour with janus green B.

Ovaries treated with 2 per cent. osmic acid for a short time.

Elsewhere (1928) the writer has laid stress on the value of treating the eggs for a short time only with 2 per cent. osmic acid for determining the shape and the nature of the chemical contents of the Golgi elements. It is true that the cell is killed by a short exposure to osmic acid, but the appearance of such a cell is almost like that of the living. This is borne out by the clear-cut experiments of Strangeways and Canti (1927), who have

shown that the cell after a short period of fixation with 2 per cent. osmic acid is almost like the living cell in vitro with regard to all the inclusions.

The first change to be noted in the appearance of the Golgi apparatus after a short period of five to ten minutes' immersion in osmic acid is that they look copper-coloured, but they still appear solid as they do in the living egg. After half an hour's

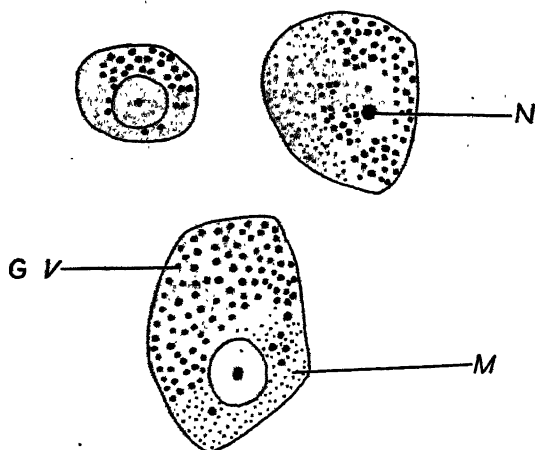


TEXT-FIG. 10. $\times 280$.

treatment with osmic acid, however, a very important change comes over the appearance of the Golgi elements, a change that gives a definite clue to their morphology. Each element now shows a dark rim and a lighter central area (Text-fig. 9). From this one has to conclude that the Golgi element is not a solid or semi-solid body, but is really a vesicle with a definite osmiophilic rim and a hollow interior.

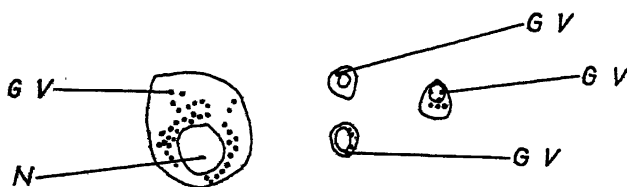
Experiments with the centrifuge were performed to prove

the entire absence of albuminous 'yolk-droplets' which, according to Harvey, are present in the egg of *Lumbricus*. The centrifuge used in these experiments was an ordinary hand



TEXT-FIG. 11. $\times 280$.

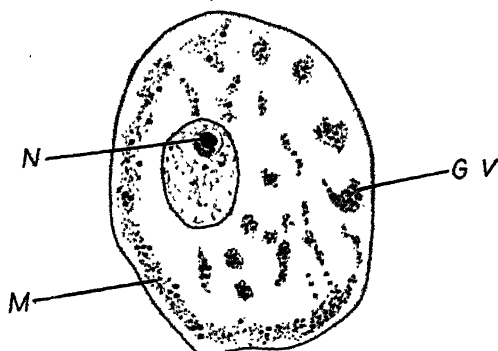
centrifuge fitted up with a motor, giving about one thousand revolutions per minute. In Text-fig. 10 are shown five oocytes



TEXT-FIG. 12. $\times 280$.

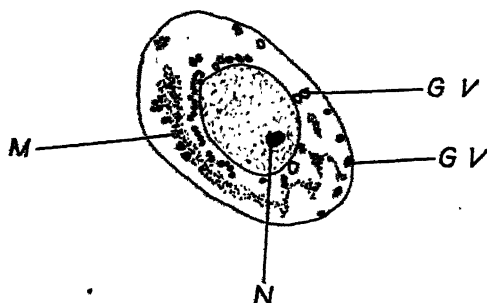
centrifuged for thirty-five minutes and kept in osmic acid for two hours. The mitochondria and the Golgi elements are completely separated into two layers, the former appearing as small yellowish granules and the latter as vesicles, each vesicle showing a definite osmiophilic membrane and a clear central substance represented by the white background of the paper. In Text-fig. 11 are shown three oocytes centrifuged for half an

hour and kept in 2 per cent. osmic acid for ten minutes only. The mitochondria appear as slightly yellowish granules, but the



TEXT-FIG. 13. $\times 560$.

Golgi elements look solid and copper-coloured as their rim is not yet impregnated. If there were any yolk-droplets of an albu-

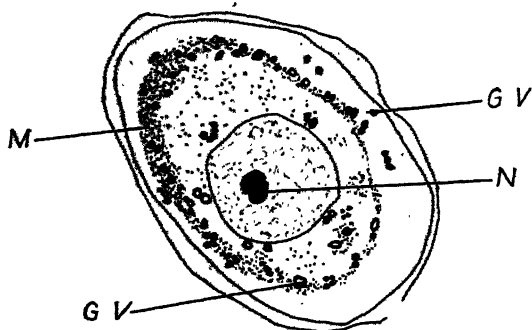


TEXT-FIG. 14. $\times 560$.

minous nature as described by Harvey it is inconceivable that they should be missed in a centrifuged egg treated with two per cent. osmic acid, for they would as usual stand out prominently as solid, white, or slightly yellowish bodies much bigger in size than the tiny mitochondrial granules and distinct from the blackened Golgi vesicles.

Fixed Preparations.

The difficulties in the way of getting first-rate preparations of the earthworm ovary are considerable. This material is remarkably refractory, and whatever the fixative used there is always a certain amount of shrinkage as shown by the tearing away of the cytoplasm from the egg membrane—a fact emphasized by Foot and Strobell (1901). Nevertheless I have been able to obtain satisfactory preparations of eggs, except the most highly advanced which lie at the distal ends of the ovarian



TEXT-FIG. 15. $\times 560$.

filaments. It is almost impossible to fix these latter in a satisfactory manner. Foot and Strobell, who made very extensive studies of the ovarian eggs of *Allolobophora* as well as of those contained in the cocoons, ascribe this refractoriness to the fact that a large number of eggs at the distal end of the ovary degenerate and are unable to develop. Luckily, however, the egg of the earthworm is such an ideal material for *intra vitam* observations that fixed preparations can perhaps be entirely dispensed with except for purposes of control.

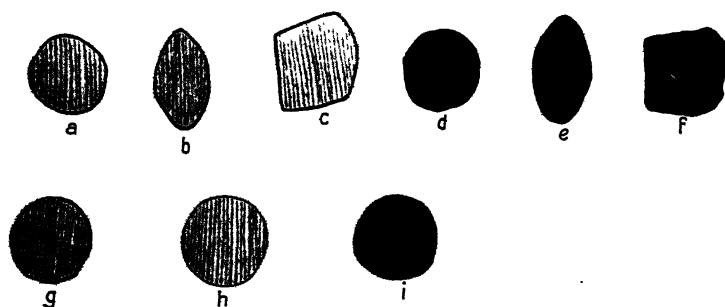
In the course of my experiments I have discovered a point of considerable importance (*vide infra*), namely, that the Golgi elements of the earthworm which are vesicular in nature contain a certain amount of fat inside them. This point has been overlooked both by Harvey (1925) and by Gatenby and Nath (1926). When the whole ovary is mounted after twenty-four

hours' fixation in Champy's fluid the Golgi vesicles appear as very black solid granules not only in the oocytes but also in the youngest oogonia (Text-fig. 12). If the same preparation is studied after about a month one is surprised to find that the Golgi elements appear as colourless or at most slightly greyish bodies. This is undoubtedly due to the decolorizing action of the xylol in the Canada balsam. When the paraffin ribbon containing Champy-fixed sections is studied the Golgi elements appear as blackened granules; but immediately after exposure to xylol to remove the paraffin the elements are decolorized and appear as colourless or slightly greyish bodies (Text-fig. 13), which are liable to be missed unless the condenser of the microscope is considerably lowered to reduce the amount of light. In whole mounts, however, the xylol of the Canada balsam will naturally take a much longer time to decolorize the blackened Golgi elements. The above observations adequately explain the statement of Gatenby and Nath (1926) that 'the Golgi elements, however, are slightly osmiophile but do not go black'.

'Kolatschev' preparations not only reveal the vesicular nature of the Golgi elements, but also adequately explain certain forms which these elements may sometimes assume. The optimum time for the proper impregnation of the Golgi elements of the earthworm egg is about four days' incubation at the temperature of about 35° C. The mitochondrial granules are usually blackened in the regions of close aggregations, but they can be very successfully bleached by potassium permanganate followed by oxalic acid.

When an optimum impregnation and proper bleaching have been secured the Golgi elements appear vesicular, each vesicle showing an intensely blackened rim and a much lighter central area (Text-fig. 16a). But more often than not the Golgi elements assume different forms, all of which must be interpreted as artifacts. In Text-fig. 16b and c the Golgi elements are more or less ellipsoid and rhomboid respectively—appearances which are due to the shrinkage inevitable in prolonged osmication. When the incubation has been carried out for about six days

most of the Golgi elements appear uniformly jet-black (Text-fig. 16*d, e, and f*). Even after shorter periods of incubation (four days) most of the Golgi elements appear solid (Text-figs. 14 and 15). It must be clearly understood that the 'solid' appearance of the Golgi elements after fixation in 'Kolatschev' is due not only to the blackening of the fatty contents of the vesicle, but also to the heavily impregnated rim, whereas the same appearance of the Golgi element after twenty-four hours'



TEXT-FIG. 16.

immersion in Champy's fluid is due solely to the reduction of osmic acid by the fat, the rim of the vesicle being not at all impregnated. It is due to this fact that the Golgi vesicles which are 'solidified' after fixation in 'Champy' are so quickly decolorized by xylol or turpentine, whereas after 'Kolatschev' these reagents fail to decolorize the Golgi elements even in months.

After a light impregnation with 'Kolatschev' the Golgi elements assume still different appearances. In Text-fig. 16*i* the rim of the vesicle has completely missed impregnation and the blackened fatty contents have been decolorized by xylol. Consequently the vesicle appears as a colourless or slightly greyish body, reminding one of the so-called 'yolk-droplets' of Harvey. In some cases, however, the rim of the vesicle is very lightly impregnated (Text-fig. 16*h*). The form of the Golgi element, which I cannot definitely explain, is a heavily impreg

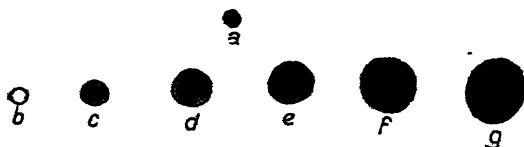
nated crescent with an osmiophobic substance attached to it (Text-fig. 16 *g*). This form results either from a partial blackening of the rim, or it represents an optical section of the vesicle.

The greatest amount of shrinkage of the egg takes place after 'Da Fano', but the Golgi elements are heavily impregnated and almost invariably appear as solid blackened granules. The mitochondria appear as much smaller granules, golden in untuned preparations and colourless or slightly greyish in the toned ones.

DISCUSSION.

The oogenesis of the earthworm is the simplest at present known. The only inclusions present in all stages of oogenesis

TEXT-FIG. 17.



a illustrates the immediate blackening of a fat-droplet in osmic acid. *b*, *c*, *d*, *e*, *f*, and *g* illustrate the gradual growth of the Golgi vesicle and the deposition of fat inside its interior as demonstrated by osmic acid.

are the mitochondrial granules and the slightly fatty Golgi vesicles. There is no yolk in the egg either fatty or albuminous. This fact is at once understood when it is remembered that the egg is nourished by an albuminous material present in the cocoon.

Thirty years ago Foot and Strobell, working with a technique much less efficient than the technique of the present day, described these inclusions in a remarkably accurate manner. A reference to the section on the previous work on the earthworm ovary will at once make it clear that the 'osmiophile' granules and the 'archoplasmic' or the 'yolk-nucleus' granules of Foot and Strobell are the Golgi vesicles and the mitochondria respectively.

According to Harvey (1925 and 1929) the mitochondria arise as a cap of threads over the nucleus, and this cap grows in size and density, migrates away from the nuclear membrane and

breaks up into its component mitochondrial threads, which become evenly spread throughout the cytoplasm of the cells. In by far the greater number of eggs of different animals studied with the most modern technique the mitochondria have been described and figured as granules. In very few cases of oogenesis have filamentous mitochondria been described. For instance, Hibbard (1928) describes filamentous mitochondria in the egg of *Discoglossus*. Indeed, so far as I am aware, this egg and that of *Lumbricus* as described by Harvey are the only eggs which are stated to have mitochondria in the form of filaments. It is also true that in tissue culture of somatic cells the brothers Lewis (1914 and 1915) have described rapid changes in the shape of the mitochondria. The granules may become arranged in a linear series or the individual granules be converted into filaments which can give rise to complicated networks.

In the egg of the earthworm, however, the mitochondria are definitely granular, both in the oogonia and the oocytes. This has been shown by Calkins (1895) and by Gatenby and Nath (1926) in the case of *Lumbricus*, and by Foot and Strobell (1901) in the case of *Allolobophora*. I am at a loss to understand the statement of Harvey (1929) that even in the fresh eggs the mitochondria are thread-like and not granular, and I am compelled to ascribe this statement to faulty technique or observation. Harvey's mitochondrial 'filaments' in fixed preparations are certainly due to the artificial alignment of granules. A similar artifact is produced in the egg of the red cotton bug. In the fresh egg and after the mitochondrial technique, namely, Flemming without acetic and iron haematoxylin, and Champy-Kull and acid fuchsin, the mitochondria appear as granules, but in some 'Kolatschev' preparations they appear as 'threads'.

According to Harvey 'a fair amount of yolk is present in the developing egg of *Lumbricus* as quite large droplets. Although not universally present in the oogonial and young oocyte stages, yet these droplets are to be found in all the stages, and it is significant that they are present in the young oogonium before the mitochondrial cap can be detected with certainty.'

'They do not go red in Champy-Kull or Bensley-Cowdry, they are yellowish or brownish in most chrome-osmium preparations, are easily decolorized by turpentine after Mann-Kopsch, do not disappear after Carnoy fixation, &c.'

Foot and Strobell, and Gatenby and Nath did not find any yolk-droplets in *Allolobophora* and *Lumbricus* respectively. I do not find them in the case of *Pheretima*, the only inclusions being the mitochondrial granules and the Golgi vesicles. If albuminous yolk-droplets were present, they should have been thrown in a separate stratum in the centrifuged eggs. What Harvey describes as yolk in his Mann-Kopsch preparations are really some of the Golgi vesicles which have not been properly impregnated by osmic acid (see Observations). What he describes as yolk in his chrome-osmium, Champy-Kull, and Bensley-Cowdry preparations are again the Golgi vesicles which have not been properly stained. Indeed it is not easy to stain them after treatment with the above fixatives on account of their vesicular nature. It is difficult to stain the rim, and unless the stain is precipitated inside them, they fail to stain. Foot and Strobell also found that the osmiophile granules very rarely react to haematoxylin (*vide supra*). The fact that 'yolk-droplets' do not disappear after Carnoy fixation does not prove that they are true albuminous yolk. In my Bouin preparations of the *Pheretima* egg granules answering to the description of 'yolk-droplets' of Harvey appear, which are really the distorted Golgi elements. Every experienced cytologist knows that the mitochondria and the Golgi elements are not always completely washed out by fixatives containing acetic acid. In many cases they remain in a very much distorted condition. I am reminded of the 'metaplasma' or 'formations ergasto-plasmiques' of earlier workers on the spermatocytes of *Lithobius*, which are really the very much corroded Golgi elements as shown by Nath (1925). Lastly, it is certainly unusual that 'yolk-droplets' should be present in the oögonia.

I am also unable to support the statement of Harvey that 'the Golgi apparatus consists of numbers of Golgi elements lying

separate in the cytoplasm', and that they 'may probably arise from the cytoplasm'. I have often seen the Golgi vesicles lying very close to each other up to the stage when they are only four in number (Text-figs. 1 and 12). From this stage onward they start dispersing, till in advanced oocytes they are more or less uniformly distributed throughout the cytoplasm. It is impossible to be certain with regard to the origin of the Golgi elements, but the fact that in the very young oogonia (Text-figs. 1, 3, 4, and 12) they lie near each other points towards the conclusion that at least in the early stages new Golgi elements rise from the division of the pre-existing ones.

Harvey (1929) is 'not convinced that only one Golgi body is present in the oogonia and youngest oocytes'. In reply, in addition to inviting his attention to Text-figs. 1 and 12 of this paper, in which only one Golgi element is shown in the youngest oogonia which are found near the septal insertion of the ovary, I wish to refer him to the excellent photographs of Foot and Strobell. Plate XLII, photo 29, shows three oocytes, 'each showing one or more osmiophile granules'.

The Golgi elements in the eggs of *Pheretima* are in the form of vesicles, each vesicle having an osmiophile rim and a clear central substance. Gatenby (see Gatenby and Nath, 1926) in the fresh eggs of *Lumbricus* also described a Golgi element as a 'somewhat irregularly spherical bead' or a 'spherule'. He described the rim of the Golgi vesicle as the 'dictyosome, or thickened edge of the Golgi bead' which 'may occasionally be seen as a highly refractive peripheral area'. In fixed preparations (Da Fano), however, Gatenby and Nath described a Golgi element as a sphere, with a thickened edge or dictyosome, which varies in shape from a perfect banana-shaped rod to an irregular curved plate. In an earlier section (see Observations) I have shown that the break in the rim of the Golgi vesicle is due either to partial impregnation of the rim, or to the 'crescents' with their sphere substance representing optical sections of the vesicles. This can be the only explanation, because in the fresh cover-slip preparations treated with osmic acid for half an hour the rim of the Golgi element is perfectly

entire. Even thirty years ago Foot and Strobell studied these Golgi elements in the living eggs of *Allolobophora* and described them as granules exactly like those which I have shown in my figures of the living eggs of *Pheretima*.

In face of the clear statement of Gatenby, that in the fresh egg of *Lumbricus* the Golgi element is a 'spherule' or a 'bead' with a 'highly refractive peripheral area', it is difficult to understand what led Harvey (1929) to make the statement 'that in the oocytes of *Lumbricus*, as shown by Nath with Gatenby (1926), the Golgi apparatus can be very easily seen in the living in the form of rodlets or crescents'. I may seem to be labouring this point, but the morphology of the Golgi element in eggs is of fundamental importance in the study of its functions.

Harvey (1929) takes exception to my statement (1928) that the Golgi elements in living eggs can be more easily studied than in the living somatic or male germ-cells because their refractive index is higher on account of the presence of colloids in the form of free fat inside them. In support of his criticism Harvey cites the egg of *Lumbricus*, and says that 'in this animal the Golgi apparatus is not concerned with fatty yolk-formation, there being no fat in the egg', although I must add that in 1925 Harvey did mention the existence of fat in this egg. In reply to this criticism I may point out that the Golgi elements of *Pheretima* can certainly be demonstrated to contain small amounts of fat if the necessary precautions are taken (see Observations), that thirty years ago Foot and Strobell demonstrated the blackening of the Golgi elements of the egg of *Allolobophora* in five minutes in 1 per cent. osmic acid, and that even in the case of *Lumbricus* Gatenby and Nath described the Golgi elements as 'slightly osmiophile', in spite of the fact that they did not take the necessary precautions. In the case of the oocytes of *Culex*, also, Nath (1929) has demonstrated the existence of fat in the Golgi vesicles by eliminating to a large extent the decolorizing action of xylol.

Gatenby and Woodger (1920), Ludford (1921), and Brambell (1924) showed that in *Patella* the Golgi dictyosomes secrete

the fatty yolk, although it is very important to note that Ludford clearly mentioned that some of the Golgi elements are directly metamorphosed into such yolk. Brambell (1924) and Nath (1924), however, demonstrated that in *Helix* and *Lithobius* respectively the Golgi element is directly converted into fatty yolk. Nath (1926, &c.), and Nath and collaborators have insisted on the fundamental morphological similarity between a Golgi vesicle and a fatty yolk-vacuole, and have shown in a variety of eggs that the latter is nothing but a swollen Golgi vesicle containing fat. Similarly King (1926) showed that 'the formation of fatty yolk from the Golgi elements in *Oniscus* is strictly comparable to the process described by Nath in *Lithobius*'.

Recently, however, Hibbard (1928) and Harvey (1929) have claimed that, in the Amphibian *Discoglossus* and the crab respectively, fatty yolk arises independently in the cytoplasm. Since I have not personally examined these forms I will neither challenge nor accept these conclusions. Reference may be made to the numerous papers by Nath and his collaborators for the details of the process of the origin of the fatty yolk from the Golgi vesicles, but I will take this opportunity to lay the utmost emphasis that I can command on three points only of great fundamental importance.

1. Hibbard and Harvey appear not to have realized that they are dealing with 'fat globules' and 'fat droplets' respectively, while I am dealing with a vesicle having a definite membrane containing fat. A simple experiment will explain my meaning and will bring home to them the difference between a fat globule and a vesicle containing fat. If particles of an oil, say clove oil, are thrown into osmic acid they go black immediately. Under the microscope they appear uniformly black and do not show an osmiophilic rim and a lighter central area. If, on the other hand, they place an advanced oocyte (about 2 mm. long) of the cockroach in 2 per cent. osmic acid and examine its contents (after rupturing the egg if necessary), at intervals from about ten minutes to forty-eight hours after immersion in osmic acid they will notice an entirely different phenomenon. After

ten minutes' immersion or even less they will notice that in addition to the solid albuminous yolk-discs (if these have appeared, because there is no definite relationship between the size of the egg and the first appearance of proteid yolk), which appear perfectly white even after many hours' osmication, there are dark-brownish vesicles of different sizes. Each vesicle shows a definite black rim (due to curvature) and a lighter brownish central area. The smallest vesicles are light brown, while the bigger are black brown, with the intensity of the brown colour increasing according to the size of the vesicles. When the osmication is prolonged, say to half an hour or more, the bigger vesicles look uniformly black without the rim (just like osmicated fat globules), showing that they contain larger quantities of fat, while the smaller vesicles, which are the Golgi elements containing smaller quantities of fat, still show a dark rim and a lighter central area. After still more prolonged osmication, as for example in Kolatshev, all the vesicles look uniformly black. The smaller vesicles can be traced back in fresh osmic acid preparations to the youngest oocyte, where they have a peri-nuclear arrangement, and in which they appear copper-coloured even after forty-eight hours' immersion. Gradually they spread out in the cell and can be demonstrated as dark vesicles after shorter and shorter periods of immersion because they are becoming more and more fatty. For further details reference may be made to Nath and Piare Mohan (1929). I have mentioned the cockroach only because it is available in all parts of the world, but the same phenomenon may be observed in the spider, in Scolopendrid, *Luciola*, and *Dysdercus*, with periods of immersion in osmic acid varying in each case. The difference between the blackening of a fat-droplet in osmic acid and a Golgi vesicle containing fat is illustrated in Text-fig. 17.

In the opinion of the writer the name 'fatty yolk' is misleading, because it is not a case of a substance *A* being metamorphosed into a substance *B*, but a case of a small vesicle simply enlarging and storing up fat inside it.

2. Let us consider those eggs in which there is admittedly no

fatty yolk, namely, the earthworm and the mosquito. Although in these eggs the Golgi vesicles do not swell up, yet they are fatty. This I have demonstrated in *Culex* (1929) and in *Pheretima* by eliminating to a large extent the decolorizing action of xylol on the blackened Golgi vesicles, and the same was demonstrated by Foot and Strobell thirty years ago in *Allolobophora*, and in spite of the fact that they did not take the necessary precautions, Gatenby and Nath (1926) found the Golgi elements of *Lumbricus* 'slightly osmiophile'.

3. In *Luciola*, Nath and Mehta (1927 and 1929) have demonstrated that the Golgi vesicles of even the primordial germ-cells contain fat inasmuch as they are blackened in ten minutes in osmic acid. In *Dysdercus*, Bhandari and Nath (in press) have shown that the Golgi vesicles are blackened in half an hour by osmic acid even in the earliest oögonia. Now a reference to Harvey's and Hibbard's papers will show that their 'fat-droplets' or 'fat-globules' appear in the cell in the course of oögenesis, while in *Luciola* and *Dysdercus* fatty Golgi vesicles (which react to osmic acid in Kolatschev or Mann-Kopsch like the typical Golgi apparatus) are present long before even the oöcyte is differentiated.

I hope Harvey and Hibbard will weigh the above conclusions carefully before they challenge the theory of the origin of fatty yolk from the Golgi elements, which I have elaborated after a prolonged study of fresh cover-slip preparations treated with osmic acid, and of the truth of which I am thoroughly convinced. Even if fat arises independently in the *Discoglossus* egg and that of the crab, as claimed by Hibbard and Harvey respectively, it does not follow that it always does so.

SUMMARY.

1. Observations on the living ovary.

The earthworm ovary, as also that of the medicinal leech, is surprisingly favourable material for the study of the Golgi apparatus and the mitochondria in the living condition. The Golgi elements stand out very prominently in all stages of

oogenesis as highly refractile spherules of a dark-greyish colour, performing a dancing movement in the cell. In the earliest oogonia situated near the septal insertion of the ovary there is a single Golgi spherule lying near the nuclear membrane. It probably divides at first into two and then into four, till in advanced oocytes there is a large number of Golgi elements distributed uniformly in the cytoplasm. The mitochondria in the earliest oogonia cannot be detected. Soon, however, they arise in the form of either a horseshoe closely fitting the nuclear membrane or a roundish mass, consisting of whitish granules, much less refractile than the Golgi elements. Gradually they spread out in the cytoplasm and perform a dancing movement. The Golgi elements and the mitochondria remain unaltered for a long time after the death of the cell.

Attention is drawn to the excellent work of Foot and Strobell (1901), who described in the fresh egg of *Allolobophora* only two types of granules, namely, the 'deutoplasmic' or 'osmiophile' granules (Golgi elements) and the 'archoplasmic' or 'yolk-nucleus' granules (mitochondria). They have also shown only one osmiophile granule in their photographs of the earliest oogonia.

2. Observations on the living stained ovary.

Neutral red and janus green B do not in any way improve the visibility of the inclusions, if indeed any improvement were desired. The Golgi elements do not at all stain with neutral red. The mitochondria may appear slightly blue with janus green.

3. Observations on fresh ovaries treated with osmic acid.

The importance of this technique is greatly emphasized. After five to ten minutes' osmication the Golgi elements become copper-coloured, but they still appear solid. After half an hour's osmication they become slightly black and each element now shows very clearly a dark peripheral rim and a clear central area. The element is therefore not a solid or a semi-solid body,

but a vesicle with a definite osmiophilic rim and a hollow interior. After two hours' osmication the vesicles become still blacker.

4. Experiments with the Centrifuge.

The centrifuge very clearly reveals the existence of only two types of inclusions, namely, the Golgi elements and the mitochondria. There is neither yolk nor any other type of inclusion.

5. Observations on Fixed Preparations.

If a Champy-fixed ovary is mounted whole, the Golgi elements appear as black granules. Within a month or so, however, they are decolorized by xylol. This proves the existence of fat inside the Golgi vesicle. In Champy-fixed sections, however, the vesicles are decolorized immediately after immersion in xylol. Kolatschev preparations demonstrate very satisfactorily the vesicular shape of the Golgi element.

6. The morphology of the Golgi apparatus in general is discussed in detail in the light of the recent work of Gatenby, Hirschler, Bowen, and others.

BIBLIOGRAPHY.

1. Bhandari, K. G., and Nath, V.—'Studies in the origin of yolk. V. Oogenesis of the Red Cotton Bug *Dysdercus Cingulatus*'. (In press.)
2. Bowen, Robert H. (1928).—"Studies on the structure of plant protoplasm. I. The osmiophilic platelets", 'Zeit. Zellf. Mikr. Anat.', 6, H. 5.
3. Brambell, F. W. Rogers (1924).—"The Nature and Origin of Yolk", 'Brit. Journ. Exp. Biol.', vol. i.
4. Calkins, Gray N. (1895).—"Observations on the yolk-nucleus in the Eggs of *Lumbricus*", 'Trans. N.Y. Acad. Sci.'
5. Foot, Katherine (1894).—"Preliminary Notes on the Maturation and Fertilization of the egg of *Allolobophora foetida*", 'Journ. Morph.', vol. ix, no. 3.
6. — (1896).—"Yolk-nucleus and Polar Rings", *ibid.*, vol. xii, no. 1.
7. — (1898).—"The Cocoons and Eggs of *Allolobophora foetida*", *ibid.*, vol. xiv, no. 3.
8. Foot and Strobell (1898).—"Further Notes on the Egg of *Allolobophora foetida*", 'Zool. Bull.', vol. ii, no. 3.

9. Foot and Strobell (1900).—"Photographs of the Egg of *Allolobophora foetida*, I.", 'Journ. Morph.', vol. xvi, no. 3.
10. — (1901).—"Photographs of the Egg of *Allolobophora foetida*, II.", *ibid.*, vol. xvii, no. 3.
11. Gatenby, J. B. (1929).—"Study of Golgi Apparatus and Vacuolar System of *Cavia*, *Helix*, and *Abraxas*, by Intra-Vital methods", 'Proc. Roy. Soc.', Series B, vol. 104.
12. Gatenby, J. B., and Nath, V. (1926).—"The Oogenesis of certain Invertebrata, with special reference to *Lumbricus*", 'Quart. Journ. Micr. Sci.', vol. 70.
13. Gatenby, J. B., and Woodger, J. H. (1920).—"On the Relationship between the Formation of yolk and the mitochondria and Golgi Apparatus during Oogenesis", 'Journ. Roy. Micr. Soc.'
14. Harvey, L. (1925).—"On the Relation of the Mitochondria and Golgi apparatus to yolk-formation in the Egg-cells of the Common Earth-worm", 'Quart. Journ. Micr. Sci.', vol. 69.
15. — (1929).—"The Oogenesis of *Carcinus Moenas* Penn., with special reference to yolk Formation", 'Trans. Roy. Soc., Edinburgh', vol. lvi, part i (no. 8).
16. Hibbard, Hope (1928).—"Cytoplasmic constituents in the Developing Egg of *Discoglossus Pictus* Otth.", 'Journ. Morph.', vol. 45.
17. King, S. D. (1924).—"Oogenesis of *Lithobius*", 'Scient. Proc. Roy. Soc.', vol. 18.
18. — (1926).—"Oogenesis in *Oniscus asellus*", 'Proc. Roy. Soc.', B.
19. Lewis, M. R., and Lewis, W. H. (1914).—"Mitochondria in tissue culture", 'Science', N.S., vol. 39.
20. — (1915).—"Am. Journ. Anat.", vol. 17.
21. Ludford, R. J. (1921).—"Contributions to the study of the Oogenesis of *Patella*", 'Journ. Roy. Micr. Soc.'
22. Nath, V. (1924).—"Oogenesis of *Lithobius forficatus*", 'Proc. Camb. Phil. Soc. Biol. Sci.', vol. 1.
23. — (1925).—"Spermatogenesis of *Lithobius Forficatus*", *ibid.*, vol. 1.
24. — (1926).—"The Golgi origin of Fatty yolk in the light of Parat's work", 'Nature', November 27.
25. — (1928).—"Studies in the Origin of Yolk. I. Oogenesis of the spider, *Crossopriza Lyoni* Blackwall", 'Quart. Journ. Micr. Sci.', vol. 72.
26. — (1929).—"Studies on the Shape of the Golgi apparatus. I. Egg-follicle of *Culex*", 'Zeit. Zellf. Mikr. Anat.', 8. Band, 4. Heft.
27. Nath and Husain (1928).—"Studies in the Origin of yolk. II. Oogenesis of the *Scolopendra*, *Otostigmus Feae* (Pocock)", 'Quart. Journ. Micr. Sci.', vol. 72.
28. Nath and Mehta (1927).—"Origin of Yolk in the eggs of *Luciola Gorhami*", 'Nature', January 1.

29. Nath and Mehta (1929).—"Studies in the origin of Yolk. III. Oogenesis of the Firefly, *Luciola Gorhami*", 'Quart. Journ. Micr. Sci.' (vol. 73).
30. Nath and Piare Mohan (1929).—"Studies in the origin of Yolk. IV. Oogenesis of *Periplaneta americana*", 'Journ. Morph.' (vol. 48).
31. Patten, R., Scott, M., and Gatenby, J. B. (1928).—"The cytoplasmic inclusions of certain plant cells", 'Quart. Journ. Micr. Sci.', vol. 72.
32. Strangeways, T. S. P., and Canti, R. G. (1927).—"The living cell in Vitro as shown by Dark-ground Illumination and the changes induced in such Cells by fixing Reagents", *ibid.*, vol. 71.

The Development of the Amphibian Kidney.

PART I.

THE DEVELOPMENT OF THE MESONEPHROS OF RANA TEMPORARIA

By

Peter Gray

(of the Zoological Research Laboratory, Imperial College of Science,
London, S.W. 7).

With Plates 27-31 and 8 Text-figures.

CONTENTS.

	PAGE
HISTORICAL SUMMARY	508
MATERIAL AND TECHNIQUE	511
DEVELOPMENT OF THE MESONEPHROS	512
A. Early units	512
B. Later units	522
i. General	522
ii. Collecting trunk	526
iii. Malpighian capsules and glomeruli	529
iv. Peritoneal funnels ¹	533
DISCUSSION	537
SUMMARY	542
GLOSSARY OF NEW TERMS	544
BIBLIOGRAPHY	544
EXPLANATION OF PLATES	545

¹ Previous workers have referred to these funnels as 'nephrostomes'. Professor Goodrich, however, has pointed out to me that this latter term is only applicable to the funnel of a nephridium. Further, in a forthcoming work he is proposing the term 'peritoneal funnel' for the 'vertebrate funnel leading from coelom to nephrocoel'. Now it is shown in the course of this research that the peritoneal funnels of *R. temporaria* lead from coelom to haemocoel, and never have any connexion with the nephrocoel; at the same time, it seems highly probable that this connexion existed ancestrally and that the funnels of *R. temporaria* may therefore legitimately come within Professor Goodrich's definition.

HISTORICAL SUMMARY.

AN extraordinary amount of confusion seems to exist at present as regards the development of the mesonephric units of Anura. Almost every worker who has worked upon the subject has not only put forward a new theory, but has, in most cases, boldly stated this theory to apply to the whole group. This would be the more easy to understand if each author had worked upon many genera; but in the majority of cases only a single genus has been investigated and there has been little or no attempt to bring the new facts observed into line with the statements made by previous authors. Though there should obviously be a general similarity in the development of the mesonephros throughout the whole group Anura, I do not suggest that the very detailed account of *Rana temporaria* which I am about to put forward is directly applicable to any other genus.

Only one previous paper, that of Marshall and Bles (1890), has been exclusively devoted to the mesonephros of *R. temporaria*; indeed, the whole genus *Rana* has been surprisingly neglected, since the two most recent papers are those of Hall (1904), who worked upon *R. sylvatica*, and Filatow (1904), who investigated *R. esculenta*. The work of Hall has been so widely quoted, and appears to have been so generally accepted, that it is proposed to use it as the base of this summary.

Hall, then, states that 'in the region of the mesonephros the mesomers detach themselves from the somite and fuse to form a continuous mesonephros blastema in which swellings are seen. These are the mesonephric blastulae—the fundamentals of the mesonephric units'. This view, first advanced by Clarke (1881), was in direct contradiction to the accounts usually accepted at this time. Fürbringer (1887) had stated that the mesonephric units arose as solid ingrowths from the peritoneal epithelium, which were joined by scattered mesenchyme cells before severing their connexion with the peritoneum and becoming differentiated into tubules. This account agreed in the main with those of Spengel (1876) and Goette (1875), save that the latter supposed

the original connexion with the peritoneum to be maintained through a ciliated funnel. The accounts given by Filatow agree in the main with those of Hall (with whose work he was obviously unacquainted) and do not merit a separate description.

Hall also confirmed the fact, already stated by Spengel (1876), Nussbaum (1880), Hoffmann (1886), Marshall and Bles (1890), and Farrington (1892), that the peritoneal funnels¹ of the adult open into the blood-system; yet even if we take this fact for granted, we find that much confusion exists as to the manner in which this connexion is formed. The account usually accepted to-day is that put forward by Hall, who supposed the developing funnel and the malpighian capsule to form the two short arms of a Y, whose base opened into the archinephric duct; the attached end of the funnel then broke away and opened into a blood-vessel. He was uncertain as to whether the lumen of the funnel was at any time in continuity with the lumen of the capsule, but inclined to the view that it was not. Spengel (who was the original discoverer of these funnels) supposed them to have had a primary connexion with the malpighian capsule, but found himself unable to trace this connexion at any stage. Nussbaum and Hoffmann merely confirmed the results of Spengel and stated themselves to be in agreement with his conclusions; the latter also pointed out (as is remarked in passing by Farrington) that the capsulo-coelomic connexion through the peritoneal funnel was maintained throughout life by the Urodela.

Marshall and Bles, working upon *R. temporaria*, pointed out that the funnels opened into the veins at the earliest stage investigated by them (20 mm.); they presumed that there must have been a connexion with the capsule at some stage of the life-history, but stated themselves unable to find this connexion. With regard to the development of the malpighian capsules, they stated that 'in tadpoles of 10 to 12 mm. length the Wolffian tubules arise as little masses of cells in the mesoblast between the aorta and the archinephric duct, a little distance from the peritoneum and quite independent of it. These masses of cells are at first segmentally arranged; they are ill-defined groups of

¹ Termed by them 'nephrostomes'.

spherical, or slightly branched cells, which rapidly acquire a more definite rod-like shape, then become tubular and, growing outwards, meet and open into the archinephric duct, while at their opposite ends malpighian bodies are formed at a slightly later date.'

We have, therefore, two distinct schools of thought as regards the original formation of the malpighian units. The first school supposes them to be derived from the peritoneum (Goette and Spengel); the second school (Clarke, Hall, and Marshall and Bles) supposes them to arise from mesodermal cells of doubtful origin. These two schools are more or less united in the work of Fürbringer, in that he regards the proliferated peritoneal cells as being joined by mesenchyme cells; both schools unite in presuming the peritoneal to be derived from some portion of the malpighian unit, from which it severs its connexion in order to open into a vein.

It has long been realized that the kidney of *Amphibia* is composed of two types of unit whose origins differ; the brief outline which we have just given applies only to the earliest set of units, termed variously the 'early units' and the 'primary units'. The development of the later units has also given rise to two distinct schools of thought. The school which regarded the first of these sets as originating in the peritoneum, supposed the secondary units (as they termed them) to be derived from buds upon the primary units and in this view they were joined by all other workers except Hall. He, however, still further divides the later units into secondary and tertiary derivatives of the blastema together with a further set of 'outer units'. The secondary and tertiary units were derived exactly as were the primary, i.e. as swellings in the solid cord of cells which he termed the blastema, but he frankly states that he is not satisfied as to the origin of the 'outer units', but suggests that they are formed by the splitting of already formed units. He makes special mention of the peritoneal funnels, suggesting that in addition to some formed by splitting of primary funnels, others might be produced from cells already in situ; from whence these hypothetical cells could have come he does not say.

The only other worker who seems to have regarded the adult funnels as presenting a special problem is their discoverer Spengel. He endeavoured to show that these funnels, about whose origin he is doubtful, maintained throughout life a connexion with some portion of a malpighian unit. He found himself, however, quite unable to prove his point, though in one case he has recorded a long ciliated tube, the free end of which was applied to the peritoneal epithelium and whose dorsal end appeared to lead to the collecting trunk. (In common with other workers he termed the 'collecting trunk', that portion of the primary tubule from which had been given off the secondary tubules.) The presence of this long ciliated tubule is of particular importance in view of the confirmation it gives to our own account of the development of these later peritoneal funnels (vide subter).

MATERIAL AND TECHNIQUE.

The tadpoles and newly metamorphosed frogs used in this work were all bred in the laboratory from *R. temporaria* obtained in the London district; post-metamorphic stages were captured in the same district. The tadpoles were graded into stages according to the length from the tip of the snout to the tip of the tail, and it was found, under the artificial conditions employed, that the following lengths, ages, and conditions corresponded.

<i>Length</i> (mm.)	<i>Age</i> (days)	<i>Condition.</i>
6-7	8	Larva just hatched from albumen.
10-12	15	External gills just vanishing.
19-22	49	
23-25	63	Hind legs just appearing.
28-30	84	Front legs evident.
10	89	Metamorphosis just complete.

These stages are singled out for special mention since they are those stages to which reference is made in the text and in the plates; a much more complete series was, of course, investigated.

The material was fixed for about 12 hours in Bouin's picro-acetic-formol and then washed in 70 per cent. alcohol till all the yellow colour was removed. Embedding, after dehydration in 90 per cent., 95 per cent., and 99 per cent. alcohols and clearing in oil of cedarwood, was carried out in 56 degrees paraffin wax and 10μ sections cut in transverse, frontal, and sagittal planes. The best stain for the sections was found to be Delafield's haematoxylin, with subsequent 'blueing' in ammonia vapour. In the differentiation of the different parts of the more advanced kidneys Pacini's triple stain was found useful, but it tends to obscure histological detail. The sections were without exception mounted in Gurr's neutral balsam.

Only one difficulty was encountered—the tendency of tadpoles from 10 to 30 mm. long to take grit into the alimentary canal; in order to obtain good sections of these stages it was found to be absolutely essential to remove the entire gut from the larva before embedding.

The reconstructions were carried out by ordinary graphic methods and subsequently shaded, with the aid of the actual sections, to simulate relief.

DEVELOPMENT OF THE MESONEPHROS.

A. Early Units.

If we examine the mesonephric region of a 10 mm. tadpole, we notice that there is an irregular mass of cells occupying a tract of the retro-peritoneal tissue along the dorso-medial wall of the archinephric duct; this tract of tissue is the 'blastema' of Hall (vide p. 508). It seems to me to be highly improbable that it should, as he states, have been cut off as a solid mass of cells at the time of the division of the mesoderm into dorsal and ventral portions, and I am far more inclined to Fürbringer's view that it is an agglomeration of specialized mesenchyme cells; that these mesenchyme cells represent that portion of the mesoderm known as the intermediate cell-mass is, of course, obvious, since in every known vertebrate it is from this portion of the mesoderm that the excretory system arises.

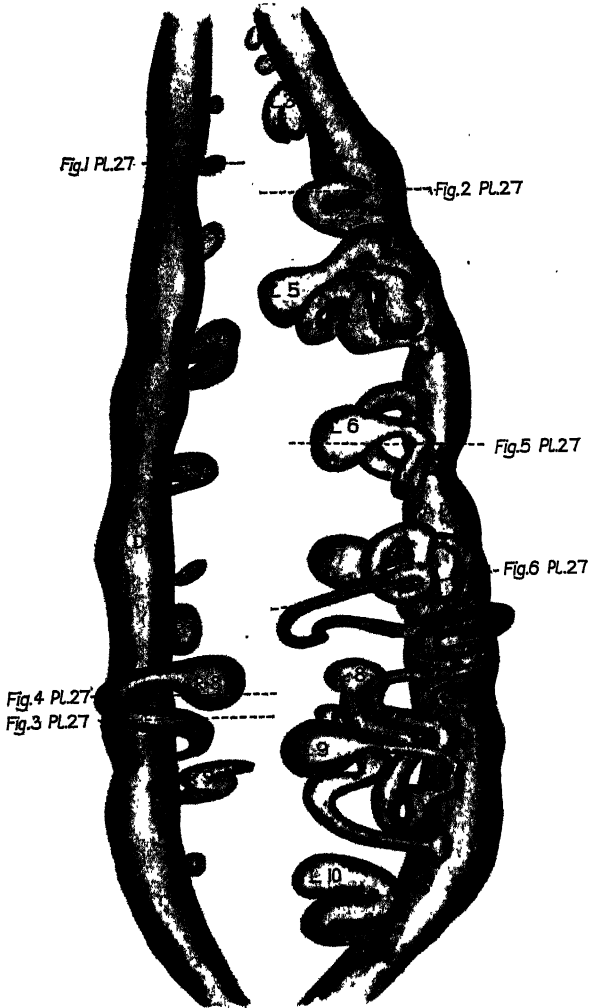
The development of the early units from this blastema is essentially as described by Marshall and Bles ; but since their account occupied less than a hundred words, very little excuse seems to be necessary for presenting a more detailed description.

A remarkable fact, for which I can find no previous mention, is that the early units develop asymmetrically on each side of the animal. In every case which I have examined, the mesonephric units of the right side are far less developed than those of the left side. It is true that all the larvae examined by me are from the London district, but I do not consider it possible that such a condition should be merely a local abnormality, and I am at a loss to explain why the fact has not been recorded by earlier workers. This asymmetry is of the utmost value to the investigator since it permits of the tracing of the whole history of the unit in a single tadpole. Text-fig. 1, for example, is a complete reconstruction of both mesonephroi from a 17 mm. tadpole ; every stage in the development, from the first nephroblast vesicle (vide subter for the explanation of this term) to the much-coiled unit, is there shown, but in order to get a better idea of the early development it is necessary to discuss, first of all, the condition in a rather younger tadpole.

The examination of a series of sections through the mesonephric region of a 13 mm. tadpole shows that a series of condensations have taken place in the mesonephric blastema—for reasons which will later become apparent I propose to term these condensations the 'nephroblast vesicles'.¹ Each consists, on the average, of about seven or eight cells, loosely pressed together and whose position bears no constant relation to the segmentation of the animal, since there are some six or seven such vesicles extending over the space of five or six segments. The vesicles are in no way joined one to the other, but are quite distinct condensations within the main irregular mass of the blastema ; it must further be clearly understood that the number of cells entering into these condensations form only a quite small proportion of the total number of cells in the blastema, and the nephroblast vesicles of *R. temporaria* are not,

¹ Vide 'Glossary of New Terms' on p. 544.

TEXT-FIG. 1.



Reconstruction of both mesonephroi of a 17 mm. tadpole, as seen from the ventral surface. *AD*, archinephric duct; *L 1* to *L 10*, malpighian units of the left side; *R 1* to *R 10*, malpighian units of the right side. (The unit *L 5* has been slightly displaced in a posterior direction in order to permit the unit *L 4* to be seen.)

therefore, identical with the 'blastulae' of Hall which were stated to be regularly placed swollen nodes connected by a smooth cord of cells.

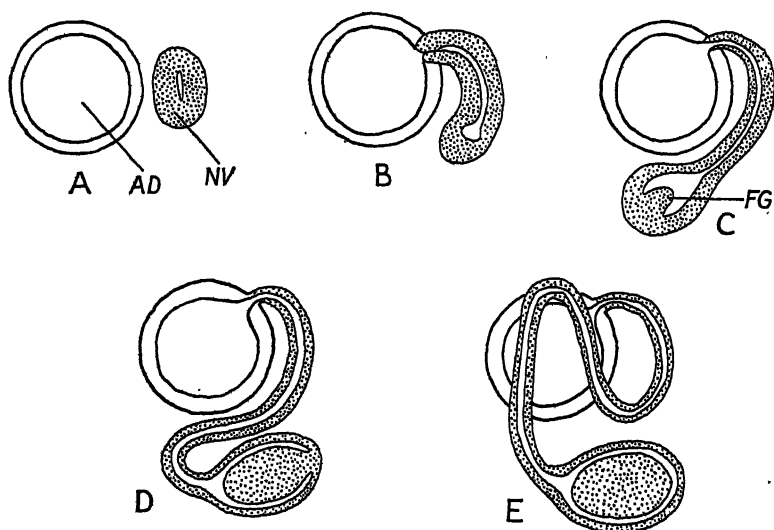
All the nephroblast vesicles arise about the same time but develop rather more rapidly in the posterior than in the anterior region; it has frequently been stated, in this connexion, that the mesonephros forms in a regular manner from the posterior to the anterior. This is a misuse of the word 'form': the fundaments of all the vesicles are formed at the same time, but they develop rather more rapidly in the posterior region. This statement is entirely relative, for a glance at Text-fig. 1 shows that there is no uniform gradation in stage from the most anterior to the most posterior unit. The right mesonephros (shown on the left-hand side of the figure) has the eighth unit (*R 8*) most highly developed, while in the left hand the seventh, eighth, and ninth units (*L 7*, *L 8*, and *L 9*) are at an almost equally advanced stage of development; again, the fifth unit on the left kidney (*L 5*) is far in advance of the more posteriorly placed sixth unit (*L 6*).

When the nephroblast vesicles are first formed they are roughly spherical in shape (*L 1*, *L 2*, *R 1*, and *R 10* on Text-fig. 1, for example), but soon become oval (*R 2*) owing to the elongation of the cells of which they are formed. This oval vesicle then develops a lumen and commences to grow rapidly at each end. The end nearest to the archinephric duct forces its way into the wall of the latter so that the lumen of the vesicle becomes continuous with that of the duct; this is diagrammatically represented in Text-figs. 2 A and 2 B. While this connexion is being formed the other end of the oval vesicle is growing downwards round the wall of the archinephric duct; the proliferation of cells at this growing tip, moreover, has not only caused the lengthening of the vesicle but has also given rise to a slight dilatation of the tip, so that at this stage the developing unit presents the subclavate shown at *R 2*, Text-fig. 1. A section which is transverse to the archinephric duct will, at this stage, cut the tip of the developing unit obliquely; such a section is shown at fig. 1, Pl. 27, and it will be noticed that the

nephroblast vesicle (*n.v.*) is still surrounded by blastema cells (*b*) and does not, as stated by both Fürbringer and Marshall and Bles, lie freely in the connective tissue.

So far only the cells comprising the two ends of the developing vesicle have increased in number, but after the connexion with

TEXT-FIG. 2.



Series of diagrams representing the formation of an early unit from a nephroblast vesicle. *AD*, archinephric duct; *FG*, fundament of glomerulus; *NV*, nephroblast vesicle.

the archinephric duct has been formed, the cells of this end remain quiescent while those of the central and terminal portions continue to divide rapidly, so that the vesicle becomes elongated into an embryo tubule; but the cells of the central portion do not divide equally on both sides of the duct and the terminal portion becomes bent back upon the remainder into the form shown at *L 4*, Text-fig. 1. The cells of the tip, however, continue to divide evenly and rapidly so that a mass of cells is formed which, upon the inner side, soon commences to push inwards and obscure the lumen; this mass is the rudiment of

the malpighian glomerulus and is represented at *FG*, Text-fig. 2 c. This ingrowing portion continues to increase rapidly in size (Text-fig. 2 d), its cells become reorientated and it finally breaks away from the capsule as the completed glomerulus (Text-fig. 2 e). This breaking away is not entirely complete, but a narrow strand, representing the blood-connexion, remains in connexion with the capsule; finally, the increase in size of the glomerulus stretches the walls of the capsule until they assume the membranous condition shown in figs. 4 and 5 on Pl. 27.

While this differentiation has been going on within the capsule, the increase in length of the tubule has carried the malpighian capsule at its tip into a position alongside the peritoneal wall of the kidney; the continued division of the tubule cells can therefore no longer give rise to an increase in length, but must result in coiling. This coiling, which is perfectly regular, takes place in the following manner.

The first bend, as already stated, is such as to cause the whole unit to become recoiled upon itself. The second is similar, but in a different direction, to the first, so that an 'S'-shaped bend is produced; this bend is, of course, the well-known 'Henle's loop', and is extremely well illustrated by the condition of the units *R 8* and *L 10* in Text-fig. 1. The distal (that is to say, farther from the archinephric duct) curve of the S bend now sends out a loop parallel to the plane of the archinephric duct; the resultant form, which is far easier to figure than to describe, is shown by the unit *L 6*. The section in fig. 3, Pl. 27, is across the region indicated on *L 6*, Text-fig. 1, and shows the rapid cellular division from which this bend takes its form. The proximal curve of the original S now pushes rapidly towards the median plane of the animal, and since there is little, or nothing, to impede its growth, it pushes farther than the bend last described so that this medially directed loop becomes rather more drawn out, and in consequence rather thinner, than the other portions of the tubule; this thin loop is well shown in fig. 6, Pl. 27, and in the unit *L 7* in Text-fig. 1. The remaining central portion of the original S now elongates and becomes bent into the form of the Greek letter Σ ; these bends are shown in

the units *L* 7 and *L* 9, Text-fig. 1. Beyond this point the coiling becomes quite irregular, since the units have now attained such proportions that they tend to press one against the other with a resultant modification of the position of their coils.

It will be noticed, on reference to Text-fig. 1, that the anterior vesicles are shown as lying against the dorso-medial wall of the archinephric duct while the more developed posterior units are shown as inserted ventrally; this is accounted for by the 'rotation' of the archinephric duct. We place the word rotation between quotation marks since this word is far more applicable to the result than to the means by which it is produced; actually the cells of the dorsal wall of the duct divide so that the area occupied by this wall is increased and, without any actual movement of the duct, the apparent orientation of its walls is thus altered, what was originally dorso-medial being pushed round until it becomes ventral. This apparent rotation of the duct continues until the point of insertion of the original units comes to occupy the lateral wall; the significance of this change becomes apparent when we deal with the development of the later units.

Now let us turn our attention to the development of the peritoneal funnels of the early units; these are not shown in the reconstruction since they would merely serve to obscure the outlines of the units.

When the growing tip of the unit reaches the peritoneum there is found to be a small roundish mass of cells lying between the two; these cells are the rudiment of a funnel. Whether these cells are blastema tissue which has been pushed down along with the tip of the unit, or whether they are a product of this latter, it is impossible to say. I am inclined to think that the former is true and that the funnel is therefore differentiated from the rest of the units at the very commencement of its life-history; at any rate, it may be quite definitely stated that this mass of tissue lies at the tip of the developing malpighian capsule and never, in *R. temporaria*, occupies the position indicated by Hall for *R. sylvatica*. This mass, which is shown diagrammatically at *FN*, Text-fig. 3 A, then becomes

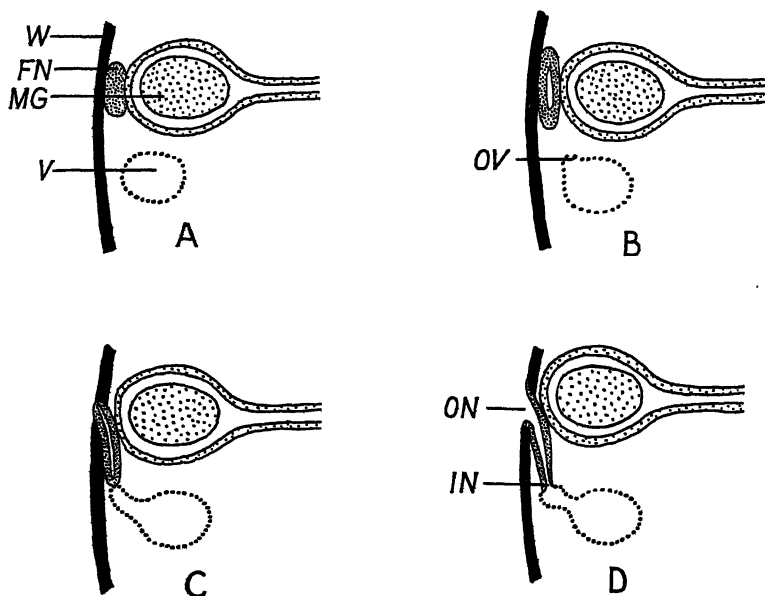
elongated into a spindle-shaped tubule, closed at both ends ; this is represented in Text-fig. 3 B. The plane of this spindle-shaped tubule is parallel to that of the peritoneum—that is to say, transverse to the plane of the long axis of the malpighian capsule ; we speak in terms of planes since the actual orientation within this plane appears to be a matter of chance. Both Hall and Marshall and Bles lamented the fact that, in the words of the latter, ‘ transverse sections do not cut the nephrostomes in a favourable plane for observation ’. This is perfectly true—but it would be equally true were the statement made of frontal or sagittal sections. There is absolutely no means of foretelling in what plane any particular funnel will lie ; it is largely this fact which causes me to believe that the funnel rudiment is early differentiated from the malpighian capsule ; for if it were the product of the latter, one would expect to find some form of constant relation between the two. A transverse section of the spindle condition is shown at *f.n.*, fig. 2, Pl. 27, and it will be noticed that the developing funnel is only very loosely applied against the wall of the malpighian capsule.

The lumen of the spindle now increases in size, as do the cells of its wall, these latter acquiring cilia. The lumen then forms a connexion with the coelom and with an outgrowth from a neighbouring vein. Fig. 4, Pl. 27, shows the connexion (*o.n.*) between the coelom and the funnel, while fig. 5 on the same plate shows the connexion of a funnel with a blood-vessel (*b.v.*) ; both these connexions appear to be formed about the same time. There is no doubt whatever that the lumen of the funnel is never in continuity with that of the malpighian capsule ; for, as we have already stated, the lumen of the developing funnel lies in a plane at right angles to that of the developing malpighian capsule, so that this latter only comes in contact with the central portion of the funnel wall.

Now so far we have only traced the development of the vesicle ; the cells of the blastema develop as rapidly as do those of the vesicle. Thus in fig. 1, Pl. 27, the blastema (*b*) consists of a few cells lying around the nephroblast vesicle. A section inter-

mediate between two vesicles would show a solid blastema mass occupying the same area as is occupied, in the section mentioned, by both the vesicle and the blastema put together; in short, as has already been pointed out, but cannot be too heavily em-

TEXT-FIG. 3.



Series of diagrams representing the formation of a peritoneal funnel from a mass of blastema lying between the wall of the malpighian capsule and the periphery of the kidney. *FN*, fundament of funnel; *IN*, inner opening of funnel to blood-vessel; *MG*, malpighian glomerulus; *ON*, outer connexion of funnel; *V*, blood-vessel; *W*, periphery of kidney.

phasized, they are not condensations of the blastema, but in the blastema.

As the units develop, therefore, they are always surrounded by the growing blastema; but the increase in the space occupied by the growing units tends to separate the main mass of blastema from the region of the archinephric duct, as is shown in

the series of sections, figs. 1 to 6, Pl. 27. In figs. 1, 2, 3, and 4, Pl. 27, the archinephric duct (*a.d.*) still retains a connexion with the blastema, but in 5 a tubule has forced its way up between the two, and in 6 two tubules occupy this position. The blastema does not become separated from the archinephric duct along its entire length, but remains connected to it by five or six 'straight tubules' whose origin is as follows.

An examination of fig. 4, Pl. 27, shows, at the dorso-medial angle of the archinephric duct, a small mass of cells (*f.st.*) which at first sight appears to form a nephroblast vesicle; this mass of cells is the rudiment of one of the straight tubules. Each develops as if it were a nephroblast vesicle up to the point at which the connexion with the archinephric duct is formed. At this stage, it will be remembered, the developing unit has the form of a short tubule whose end is swollen in a manner suggestive of a malpighian capsule; but in the case of the straight tubule this malpighian capsule never becomes perfected, neither does the tubule ever become coiled. It remains as a straight connexion between the archinephric duct and the blastema mass, increasing in length as the distance between the two increases. The development of this straight tubule is diagrammatically shown in Text-figs. 6 A to 6 C, and will be given in more detail when we come to discuss the development of the later units.

To sum up, then, the development of the kidney from 12 to 17 mm., we may say that about (varying in different individuals) ten units are formed upon each side, each unit being derived in its entirety from a nephroblast vesicle, which is itself a condensation in the blastema. At a slightly later date five or six vesicles arise which do not develop into perfect units, but remain as straight tubules running from the dorso-medial blastema to the dorso-lateral archinephric duct. Further, the peritoneal funnels, which never have any connexion with the lumen of a malpighian capsule, develop from masses of tissue lying between the malpighian capsule and the peritoneum and form a clear connexion between the coelom and the blood-system.

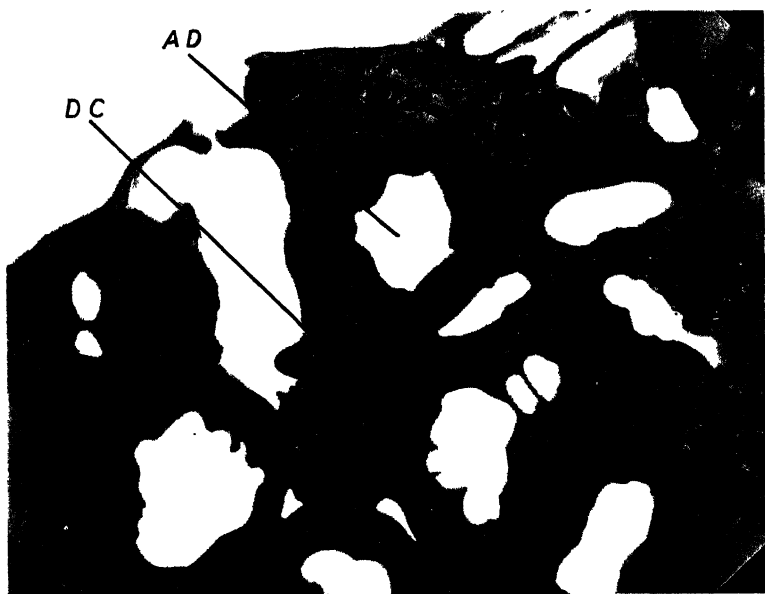
B. Later Units.

i. General.—The development of what I propose to term 'later units' (secondary, tertiary, and outer units of Hall) commences at about 18 mm. and continues up to about two years. The processes which are about to be described have been worked out from sections of larvae varying between these two ages, but the actual sections figured are for the most part from 20 mm. and newly metamorphosed tadpoles. These two stages have been selected solely because the development of the units appears to be carried on most actively at these times; almost any stage in the development of either a malpighian unit or a peritoneal funnel may be found in any specimen between the two ages mentioned.

In order clearly to understand what follows, it is essential to realize the general structure of the mesonephros of a young tadpole; Pl. 28 shows photographs of transverse (fig. 7, Pl. 28) and obliquely parasagittal (fig. 8, Pl. 28) sections of a 20 mm. stage. The asymmetric condition of the mesonephros is very clearly shown. The blastema (*b.*) occupies (in fig. 7, Pl. 28) the dorso-medial angle of both mesonephroi; running down from this in a ventral direction is a 'string' of three malpighian glomeruli, of which the dorsal is the least developed, the middle one (*m.c.*) clearly recognizable, and the lowest one in an almost perfect condition. Lying against the side of this last is a peritoneal funnel whose outer opening (*o.n.*) connects with the coelom, and whose inner opening (*i.n.*) is clearly shown as leading into the large blood-vessel (*b.v.*). Following this latter round in a ventro-medial direction to the ventral margin of the kidney, we find two tubules (*nst.t.*) actually lying within the blood-vessel. Just to the left of these two tubules there is a further malpighian capsule whose glomerulus is very small and has no connexion (either in this or in any other of the neighbouring sections) with the blood-supply; from this, and from the fact that the walls of the capsule are being crushed in by the surrounding tubules, we may presume that the glomerulus is degenerating. Returning once more to the dorsal side of the

mesonephros, we see that the archinephric duct (*a.d.*) has now become pushed a considerable distance from its original position, towards the lateral margin of the kidney; the duct bears upon its ventral surface a small conical mass of cells which, when seen under a higher magnification (Text-fig. 4), shows a glassy protoplasm and irregular nuclei—which is, in fact, a typical

TEXT-FIG. 4.



Micro-photograph of region indicated in Pl. 28. *AD*, archinephric duct; *DC*, lobe of degenerating tissue attached to the duct.

mass of degenerating tissue. The remainder of the area of the kidney is occupied by large tubules whose lumina appear to be partially obscured by a fine reticulum.

Turning, for a moment, to the longitudinal section (fig. 8, Pl. 28), we see that the blastema (*b.*) (in that portion of the section in which it is cut) lies along the upper margin of the kidney as an irregular band of darkly staining cells, from which

hangs down, at one point, a string of two malpighian capsules with their glomeruli. The more ventral (and the more highly developed) of these two glomeruli is associated, at its tip, with a funnel (*n.*): At about the centre of the dorsal margin of the section there is a reorientation of the blastema cells into a form strongly suggesting a downwardly growing tubule (*nst.v.*). Owing to the plane in which the section lies, the archinephric duct is not cut at any point, but at *s.t.* there is a darkly staining duct (cut in transverse section) which may be easily identified as one of the 'straight tubules' to which some reference has already been made; growing down diagonally from the straight tubule, there is a further mass of cells (*o.s.t.*).

Thus it will be seen that the kidney at 20 mm. differs sharply from that at 15 mm. in many particulars. The malpighian capsules at this latter stage are arranged at approximately equal intervals in a longitudinal direction; at 20 mm. they appear to be arranged in a series of dorso-ventrally hanging 'strings'. Further, tubules are now present which actually lie in a blood-vessel. There also remain to be explained the lobes of degenerating cells on the archinephric duct and the apparently degenerating malpighian glomerulus.

All these differences have been remarked from the consideration of two isolated sections; let us now further investigate these differences by means of a reconstruction (fig. 9, Pl. 29). In this plate the general mass of black represents the outline of a portion of the mesonephros of a 20 mm. tadpole; on this have been superimposed the peripheral blood network (red), a malpighian capsule (yellow), the archinephric duct, and 'straight tubule' (blue), a peritoneal funnel (green), and the tubule (also green) which in the section was seen to be inside a blood-vessel. It has been thought desirable only to include one nephrostome and one malpighian capsule within the reconstruction in order to avoid the confusion which would inevitably arise were all the malpighian capsules with their intercoiling tubules shown.

It now becomes evident that what we have so far referred to as the straight tubule is the 'collecting trunk' of previous writers. Its development, which will be later given in more

detail, has already been briefly outlined, and in the example reconstructed there are no features of particular interest attaching to it. The peripheral blood network is, so far as we know, of a form common to all immature mesonephroi and presents no feature of special interest.

The malpighian unit, on the contrary, is altogether extraordinary, since a reconstruction of its tubule (also yellow) shows this latter to end blindly. I would at this point categorically state that I have never found any tubule, other than the straight tubule or collecting trunk, opening into the archinephric duct in any tadpole of more than 20 mm. length. What, then, has become of the early units which we left in an apparently functional condition at 17 mm.? This question is, in my opinion, fully answered by the little masses of degenerating tissue which, at 20 mm., we found adhering to the archinephric duct; that is to say, I regard these masses of tissue as the points at which the early units have severed their connexion with the archinephric duct. I have been fortunate enough, in one instance, to find an abnormal case (in a metamorphosing frog) in which the primary tubule had not severed its connexion. Text-fig. 5 is a photograph of this case as seen in transverse section and it will be noticed that the rapidly degenerating connexion (*DC*) is upon the opposite side of the archinephric duct (*AD*) to the straight tubule (*ST*). Now it will be remembered that the archinephric duct 'rotates' upon its axis (vide p. 518), and this one section therefore bears out the two new points which I have so far introduced into this account, viz.:

i. That the straight tubule or collecting trunk has no relation at all to the early units; since if it had it would have to be inserted upon the same side of the duct as are these units.

ii. That the early units sever their connexion with the archinephric duct; since it is well known that this duct in the adult condition runs along the outer edge of the mesonephros and the position where were inserted the early units would therefore come to lie along that wall of the duct which is farthest from the main mass of the kidney—a *reductio ad absurdum*.

There remains, in the reconstruction which we are at present

describing and in the two sections which we have already described, the 'tubules lying within the blood-vessel'. A reconstruction of this tubule (green in Pl. 29) shows it to have one extremity ending blindly in the dorso-medial blastema mass and the other end, which is internally ciliated, ending blindly just outside a blood-vessel; the coils of the tubule between these two extremities lie actually in the course of a blood-vessel. Such parts of the tubule as do not actually lie within the vessel are, in the tadpole, embedded in the general blastema which still (20 mm.) surrounds the developing tubules and therefore forms the 'interstitial tissue' of the mesonephros. The presence of this 'interstitial tissue' will be the more easily understood if we emphasize once again that the original nephroblast vesicle was surrounded by a mass of blastema and that this blastema has grown proportionally with the tubules.

To sum up, then, what we have learned from both sections and reconstructions of the mesonephros of a 20 mm. tadpole, we find:

- i. That the archinephric duct is furnished at intervals with collecting trunks.
- ii. That none of the malpighian units appear to have any direct connexion with the archinephric duct.
- iii. That the greater part of these units appear to hang down in dorso-ventrally directed strings.
- iv. That there is a curious set of tubules, ending blindly at both ends, which more or less closely follow the interior of the blood-system.

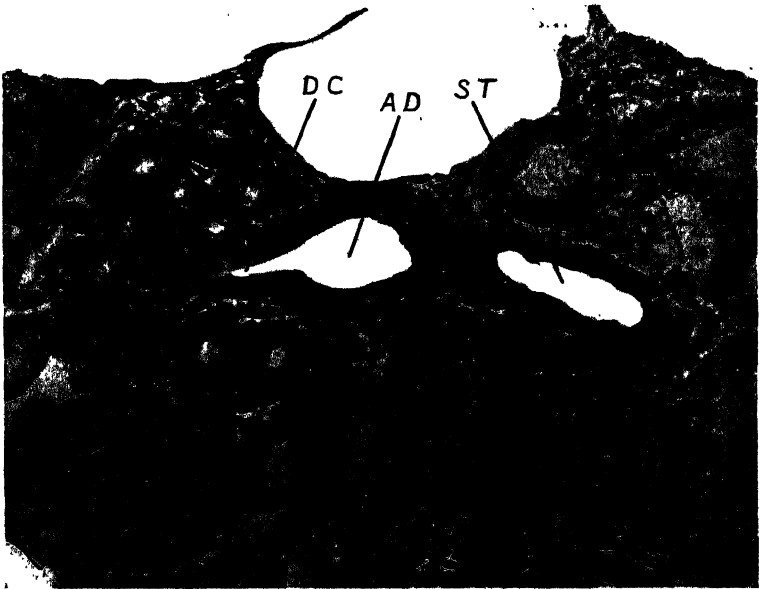
Let us examine these points in the order in which we have given them.

ii. Collecting trunk.—Though we have already, in order to link up the development of the early with the later units, given a brief outline of the history of the trunk, it would be as well to describe this in more detail.

All workers, from Fürbringer onwards, have noted the existence of the collecting trunk, but all have, without exception, supposed it to be derived from one of the early units. In this connexion it is significant to note that all these workers, save

only Marshall and Bles (who regarded the collecting trunk as an accomplished fact), have worked upon other forms than *R. temporaria*, and I am perfectly prepared to admit that the description which follows may be but another instance of

TEXT-FIG. 5.



Micro-photograph of the archinephric duct and surrounding tissue of an abnormal metamorphosing frog. *AD*, archinephric duct; *DC*, degenerating connexion of an early unit with archinephric duct; *ST*, straight tubule.

the highly modified developmental history exhibited by this form.

In *R. temporaria*, then, the first origin of the collecting trunk is a small spherical condensation in the dorso-medial blastema mass and is, at first, indistinguishable from those from which the early units are derived. These condensations usually appear at about the time when the first of the early units has attained its highest development—that is to say, at about the

stage reached in the reconstruction, Text-fig. 1. There are five or six such vesicles and there appears to be no definite segmental arrangement nor yet any relation in position to the early units. One of these vesicles, as already noted, is shown at *f.st.*, fig. 4, Pl. 27. This vesicle elongates as the archinephric duct is pushed away from the blastema and at the same time develops a swelling at its tip; fig. 15, Pl. 31, shows a section of this stage, *f.st.* being the fundament of the straight tubule. I have no doubt whatever that this straight tubule is a modified unit—modified, that is, long before it becomes in any way comparable to a unit—and we may therefore safely regard the swollen tip as the vestige of what was once a malpighian capsule. The plane of this section shows the abortive malpighian capsule as separate from the fundament of the straight tubule, as there is, in this section, a slight upward bend of the tubule at this point.

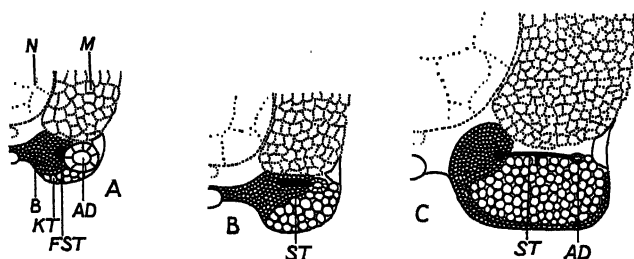
Very shortly after the stage figured has been reached the elongated vesicle acquires a lumen and forms a connexion with the archinephric duct. As this latter is pushed farther and farther from the main mass of the blastema so does the straight tubule increase in length until (fig. 16, Pl. 31) it extends across the whole width of the dorsal surface of the mesonephros. The lumen of the tubule never penetrates the abortive malpighian capsule, which retains, at any rate up to two years, the appearance of a solid ball of cells.

So much for the actual tubule; we have yet to explain the mass of downgrowing cells (*o.st.*) shown, in fig. 8, Pl. 28; as attached to it. These outgrowths appear at irregular intervals along the length of each straight tubule arising as slight swellings upon the wall of the latter. These thickenings grow outwards as buds which continue to elongate and, when they have attained a length of about twice the diameter of the straight tubule, acquire lumina which become contiguous with that of the straight tubule. This condition is very clearly shown in fig. 17, Pl. 31, where *st.* is the straight tubule and *o.st.* the bud; the abortive malpighian capsule is also shown (*a.m.c.*). This whole course of development is shown in Text-fig. 6.

We are left, therefore, with a straight tubule, one end of

which ends blindly in a mass of cells representing an ancestral malpighian capsule and the other end of which opens into the archinephric duct; at irregular intervals along the straight tubule there are buds. In order to understand the function of these buds we must first of all discuss the second point given in the summary above—the development of the malpighian capsules and glomeruli of the later units.

TEXT-FIG. 6.



Series of diagrams illustrating the withdrawal of the blastema from archinephric duct, and the formation of the straight tubule. *AD*, archinephric duct; *B*, blastema; *FST*, fundus of straight tubule; *KT*, kidney tubules; *M*, myotome; *N*, notochord; *ST*, straight tubule.

iii. Malpighian capsules and glomeruli.—The main blastema mass of a 20 mm. tadpole consists, as we have already stated, of a continuous mass of tissue along the dorso-medial angles of both mesonephroi. In this blastema there have already arisen the nephroblast vesicles, giving rise to the early units, and a set of some six further vesicles which give rise to the straight tubules.

There now arise, intermediate between, but not segmentally related to, the early units, another set of vesicles which we propose to term 'capsuloblast vesicles';¹ the explanation of this name is afforded by the fact that these vesicles give rise to the later malpighian capsules and glomeruli. Each capsuloblast vesicle, when it first arises, is identical with one of the early

¹ Vide 'Glossary of New Terms' on p. 544.

nephroblast vesicles—i.e. it is a spherical condensation of some seven or eight blastema cells. The blastema cells immediately surrounding this condensation then withdraw slightly so as to leave a small vacuole about the lower hemisphere of the vesicle. This stage is shown in fig. 10, Pl. 30, where *b.* is the blastema and *cp.v.* the capsuloblast vesicle.

The cells comprising this latter then multiply very rapidly till a solid, compact sphere of cells has been formed which may now be clearly distinguished, both by their smaller size and by their darker staining reactions, from the surrounding blastema. Such a condition is shown in fig. 11, Pl. 30, where it will be noticed that one of the central cells of the capsuloblast vesicle is actually in a condition of mitotic activity. While this increase in the number of the vesicle cells has been going on, the surrounding blastema cells have still farther withdrawn so that the condensation is now entirely cut off from the blastema save by a narrow neck of cells. This is the first stage at which the condensation may be clearly recognized as an embryo malpighian unit. The cells of the vesicle now become reorientated into a series of concentric spheres surrounding a solid inner mass. These concentric spheres are the fundament of the glomerulus; why this concentric sphere arrangement should take place I do not know. There is no suggestion of this formation in the perfect glomerulus, and indeed this form is soon lost by the continued rapid multiplication of the cells. It is at this stage (fig. 12, Pl. 30) that one begins to find traces of the formation of the tubule, in the blastema cells which form the boundary of the vacuole in which lies the capsuloblast vesicle. There is a tendency on the part of these cells to become elongated and reorientated into the form of an investing sheath (*f.k.*, fig. 12, Pl. 30) which becomes more thickened and clearly marked out as development proceeds. One portion of this sheath (that actually marked by the line *f.k.*) becomes especially thickened and later grows out as the actual tubule.

The next change observed is in the formation of the blood-supply to the glomerulus. This is formed by an outgrowth (*b.s.*, fig. 13, Pl. 30) from the cells originally comprising the

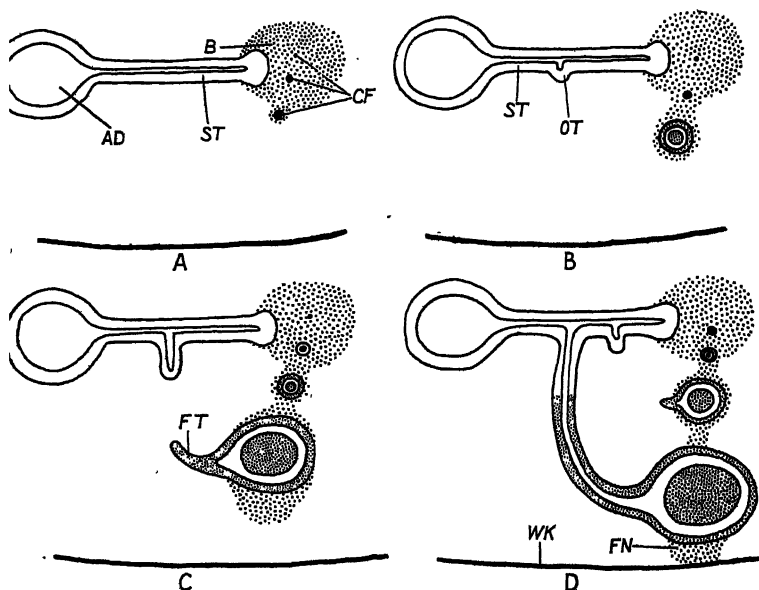
capsuloblast vesicle, which connects with a blood-vessel. At the same time as this is occurring the fundament of the tubule increases rapidly in size, acquires a lumen, becomes elongated into a regular tubular form, and commences to coil. The vacuole (which is now, of course, the cavity of the malpighian capsule) increases in size and the glomerulus assumes a typical form. The completed malpighian unit has been too well described to need re-description here, but in order to render the series of illustrations complete it is shown in figure 14, Pl. 30.

So far we have only dealt in detail with the capsule and the glomerulus; there remains the tubule. If this description has been followed, it will have been realized that the capsule and glomerulus are formed in a portion of the kidney which is now widely separated from the archinephric duct. How, then, can the connexion between the two be acquired? The answer is that as the tubule grows out it turns in an upward direction and fuses with one of the buds which has itself grown out of the straight tubule. Thus a mental picture of the malpighian units at this stage shows us a dorsally running transverse straight tubule from which hangs by the malpighian tubule, as fruit hangs from a branch, a malpighian capsule.

Now all this description has dealt with the formation of a single unit and tubule; we have not yet furnished any explanation of the phenomenon previously remarked—that the capsules and their contained glomeruli appear to hang down in ‘strings’ from the dorsal blastema. The explanation of this phenomenon is that the capsuloblast vesicles do not arise irregularly throughout the whole length of the blastema but only in certain definite tracts, each tract being apparently capable of giving rise to an indefinite number of units. As each capsuloblast vesicle develops, the growth of the blastema mass above it causes it to be pushed ventralwards; thus a clear tract of blastema is formed immediately dorsal to the vesicle and in this tract a further vesicle arises, which is in turn pushed downwards. This second vesicle is now pushed downwards to make room for a third, the third pushed down to make room for a fourth, and so on. The development of the individual vesicle appears to take place at such a

rate that the unit assumes its final form at the time when it has reached the ventral wall of the mesonephros; this rate of development is therefore variable, since the older the mesonephros, the farther will the vesicle have to be pushed before

TEXT-FIG. 7.



Series of diagrams representing the formation of the later malpighian units. *B*, blastema; *AD*, archinephric duct; *CF*, capsuloblast vesicles; *FN*, fundus of one of the later funnels; *FT*, fundus of malpighian tubule; *OT*, bud from straight tubule; *ST*, straight tubule; *WK*, ventral wall of kidney.

it reaches the ventral wall. Thus in Pl. 28 the mesonephros shown in transverse section is rather more advanced than that shown in longitudinal section; in both kidneys the ventral capsule is at the same advanced stage of development, but in the younger example the string is only composed of two capsules while in the elder it is composed of three.

The course of development which we have just described is summed up diagrammatically in Text-fig. 7. The capsuloblast

vesicles (*CF*, 7 A) arise in the blastema (*B*, 7 A), each vesicle descending as it develops and further vesicles arising above it. An outgrowth (*OT*, 7 B) appears on the straight tubule (*ST*, 7 B), and the developing capsule sends out a tubule (*FT*, 7 c) which meets and fuses (7 D) with this outgrowth.

It will be noticed in 7 D that the lowest malpighian vesicle does not actually lie in contact with the ventral wall of the kidney (*WK*, 7 D), but is separated from it by a small mass of blastema (*FN*, 7 D); this mass of blastema is the fundament of one of the later peritoneal funnels, to whose development we will now proceed.

iv. Peritoneal funnels.—There are two quite distinct methods of formation of the later funnels. The first method, which is only found in quite young (20 to 22 mm.) tadpoles, is analogous with the formation of the funnels of the early units. Each string of malpighian capsules bears at its tip of blastema cells which, when they reach the wall of the kidney, reorientate themselves into a spindle which acquires a lumen and later opens both from the coelom and to a blood-vessel. Text-fig. 3, in which is diagrammatically shown the development of the peritoneal funnel of an early unit, displays with equal accuracy the changes which take place in the course of the formation of one of these later funnels.

Now it is obvious that the funnels produced in this manner will be numerically fewer than the malpighian units; but it has been frequently recorded that the funnels in an adult kidney outnumber the malpighian units by three or four to one. Two suggestions have so far been advanced to account for these later funnels: (i) that they are formed in situ from the wall of the peritoneum, or (ii) that they are produced by the division of already existing funnels. I have observed nothing in any of my sections which in any way bears out either of these suggestions; in my opinion the peritoneal funnels are produced in the following manner.

If we revert, for the moment, to our examination of the blastema of a 20 mm. tadpole, it will be remembered that not only are there capsuloblast and straight tubule-forming vesicles

produced, but also a further set of condensations (of which one is shown at *nst.v.*, fig. 8, Pl. 28) for which we have so far recorded no function. This vesicle (which for reasons later becoming apparent will in future be referred to as the vesicle of a 'funnel-forming tubule')¹ develops in a manner utterly different from that of any of the other vesicles.

The original spherical shape of this vesicle soon becomes modified to that of a hemisphere by the excessive growth of one side of the vesicle and the consequent crushing of the other side against the upper margin of the kidney; this hemispherical condition is apparent in the section already quoted. The rapid growth of the lower side then continues so that the vesicle assumes the form of a blindly ending tubule; this slightly later stage is represented at *nst.v.*, fig. 10, Pl. 30. The tubule grows rapidly in length, pushing downwards towards the lower margin of the kidney; shortly before it reaches this margin, the tip of the tubule enters the peripheral blood-system by forcing its way through the inner wall. We have here, therefore, the explanation of the tubules which have already been noted as lying within this blood-system. The sole function of these tubules is to give rise to peritoneal funnels; since both the existence of these specialized tubules and the function subserved by them is here noted for the first time, it is obvious that a name must be found to describe them and we propose to employ the term 'funnel-forming tubules'.¹ The actual method of funnel production from these tubules is as follows.

When the tip of the tubule has accomplished about half of its journey towards the peripheral blood-system, it becomes internally ciliated; by the time that the tip has penetrated the blood-vessel the whole lower third of the tubule has acquired these internal cilia. Shortly after its entry into the blood-system the tip of the tubule lays itself parallel to the outer wall of the blood-vessel—that is, to the outer wall of the kidney. This orientation of the tip is shown in fig. 18, Pl. 31, where *nst.t.* is the ciliated tip. That wall of the tubule which lies against the periphery of the blood-vessel then breaks

¹ Vide 'Glossary of New Terms' on p. 544.

down (at the point *bn.*, in fig. 19, Pl. 31), and in this is soon followed by the inner wall of the tubule; in short, the whole tip detaches itself from the tubule and lies against the outer wall of the kidney. This wall is not, as it is usually figured, composed of a thin sheet of squamous epithelium, but is strongly reinforced in many places by agglomerations of the blastema cells; it is to these blastema cells (*b.*, in the two figures already quoted) that the now severed tip becomes attached. This severed tip, which, it will be remembered, is internally ciliated, may now be termed a ciliated funnel.

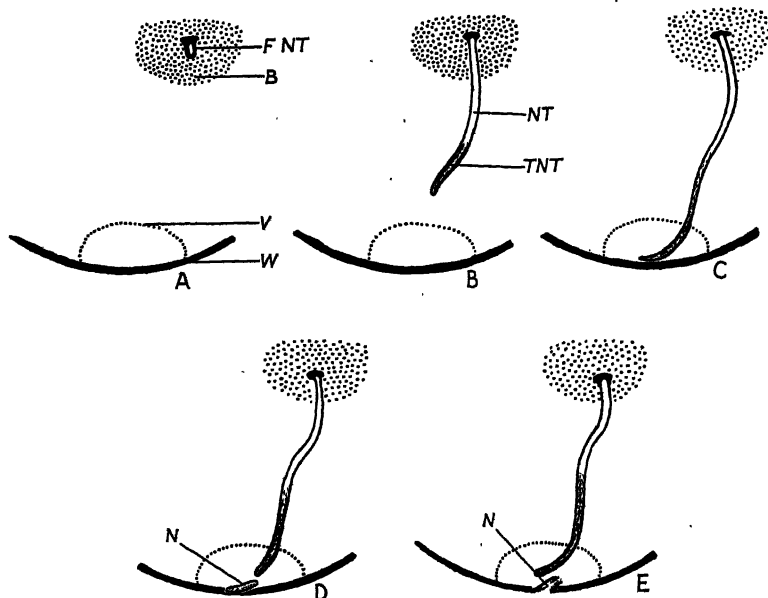
We have then, at this stage, a funnel which opens internally to a blood-vessel but whose outer extremity ends blindly in a mass of blastema cells. This blindly ending tip then forces its way to the surface of the kidney where, as in the case of the funnels produced by other methods, a coelomic connexion is formed.

Now let us return to the funnel-forming tubule. The end, from which has been separated the funnel, soon heals, but even before this healing has taken place there is an exceedingly rapid growth of the cells comprising the wall of the tubule which lies farthest from the periphery of the kidney; this rapid growth causes the tubule to turn sharply back upon itself, as is shown in the tubule reconstructed on Pl. 29 where that portion of the tubule most nearly approximated to the funnel presents a 'V'-shaped formation. This 'V' shape is very typical, being present in every funnel-forming tubule which I have reconstructed and forms, in my opinion, one of the most convincing arguments in favour of the method of funnel production just described. For there appears to me to be no good reason to account for a sudden directional change in an otherwise smoothly coiled tubule, other than that the tubule has been damaged at the point at which this directional change occurs; such damage is more than sufficiently accounted for by the detaching of the entire tip.

The typical appearance presented in section by a newly formed funnel and its attendant funnel-forming tubule is shown in the series of sections (figs. 20 to 22) on Pl. 31, which are taken at about 25μ intervals from a uniform series. Fig. 20, Pl. 31,

shows the internal opening of the funnel to the blood-vessel; fig. 21, Pl. 31, shows this funnel running through the centre of a mass of blastema (*b.*) from whose edge the funnel-forming tubule (*nst.t.*) has not yet become entirely separated; fig. 22,

TEXT-FIG. 8.



Series of diagrams illustrating the formation of a later peritoneal funnel. *B*, blastema; *FNT*, nephrostoblast vesicle; *N*, funnel; *NT*, funnel-forming tubule; *TNT*, ciliated tip of funnel-forming tubule; *V*, peripheral blood-vessel; *W*, ventral wall of mesonephros.

Pl. 31, shows the external opening of the funnel from the coelom as well as the apparently double funnel-forming tubule, this latter having been cut just behind the tip of the 'V'-shaped bend.

Each funnel-forming tubule gives rise to a large number of funnels; for obvious reasons it is impossible to say exactly how many. It is also, unfortunately, impossible to say for how long these tubules continue to exist. They first appear about

20 mm. and are both present and extremely active at metamorphosis; beyond this stage it becomes almost impossible to trace any individual tubule through a number of sections, and though I can quite definitely state that I have found no trace of these tubules in a frog about 18 months old, I would not care to be held responsible for the statement that they are not then present.

The method of funnel production which has just been described is summed up diagrammatically in Text-fig. 8. 8 A shows the vesicle of the funnel-forming tubule (*FNT*) lying in the blastema (*B*) and already commencing to grow downwards towards the lower wall (*W*) of the kidney, against which lies the blood-vessel (*V*). 8 B shows the ciliated tip (*TNT*) of the tubule (*NT*), while in 8 C this tip has penetrated the blood-vessel and arranged itself parallel to the outer wall. At 8 D the tip has become detached as a funnel (*N*), which latter is shown in its final condition in 8 E.

To sum up, then, all that we have learned of the formation of the later funnels, we may say that:

- i. There are two methods for the production of such funnels;
- ii. The first set arise one at the end of each string of malpighian units;
- iii. The second set are produced from tubules modified to subserve this function;
- iv. No peritoneal funnel ever opens into a malpighian capsule.

DISCUSSION.

It would seem at first sight that the results here published differ widely from those of previous writers; but if a more thorough comparison be made, it will be found that such new facts as have been introduced into this description serve to fill some of the more obvious gaps left in former works. Let us, then, take each point in the order in which it has been made and contrast it with other accounts.

We come, first of all, to the early units. These develop in a manner common to many vertebrates and which has already been roughly outlined for *R. temporaria* in the work of

Marshall and Bles; that is to say, cells which represent the intermediate mesoderm form an agglomeration which acquires tubular form, becomes connected to the archinephric duct, coils in a regular manner, and acquires a malpighian glomerulus.

With relation to the coiling, it is of great interest to compare some of my reconstructions with those published by various authors for various forms of fish. It will be found that the stage figured at *L 6*, Text-fig. 1, is common to both fish and *Rana*; whether any significance can be attached to this point will be discussed later. Our account, therefore, of the early units contains only one entirely new point—the method of formation of the peritoneal funnels. The remainder of the description only serves to give in detail what has already been roughly sketched by Marshall and Bles.

The development of the later malpighian units, however, is not clearly analogous to the same process in any other vertebrate; but it is, none the less, quite easy to see how this development has been evolved. The entire crux of the matter, in my opinion, lies in the early separation of the blastema from the archinephric duct and the need for great speed in production due to the very short larval life.

We may, I think, take it as axiomatic that a vertebrate malpighian unit arises, in some way or another, from the intermediate mesoderm; when this mesoderm lies closely applied to the archinephric duct, the most economical—but at the same time the slowest—method of production is that shown by the early units. When the intermediate mesoderm, however, has become separated from the duct it would entail a great waste of time were each unit to send out a connexion to the archinephric duct across the whole breadth of the kidney; hence the early specialization of a unit to the function of collecting trunk.

It is stated (and with a great deal of confirmatory evidence) that in *Urodeles* this trunk is in itself a functional unit from which the later units are derived as buds. If this is so, then our description merely bears out what has already been frequently remarked—the great shortening of developmental processes shown by *Rana*; for if our description has been followed it

will be quite obvious that the straight tubule is, as shown by the abortive malpighian capsule at its tip, merely a unit which becomes modified at an early, instead of at a late, stage of its existence. That the malpighian capsules and glomeruli should be formed from a separate piece of blastema and not in any way from an existing tubule is a modified condition which finds no exact parallel in our present accounts of other Amphibia.

I consider, therefore, that the later units of *Rana* are directly comparable to what has been described as the whole history of the mesonephros in Urodeles. How, then, can we account for the early units of *Rana*? It seems a perfectly fair assumption that they represent an ancestral condition—a condition where a longer larval life had not rendered speed an essential factor in production. This theory is rather borne out by the great similarity shown in the method of coiling of these tubules and of fish kidney tubules. It is a well-known fact that all vertebrate kidney tubules show the familiar 'S' bend (Henle's loop); it is difficult to see why this form should have been adopted by so many animals unless we assume it to have been the form adopted by the very earliest ancestors of the group. Now if we accept the view that one type of bend has been retained from what may well be termed a primordial ancestor, we should have very little trouble in believing that other regular bends, common to the fish and to an early amphibian kidney, form evidence of a relationship between the two.

There is one point arising out of this argument which is a little difficult to understand—the apparent absence of this early set of units from the Urodelan kidney. The word apparent is employed since it seems very probable that a thorough re-investigation of this type may show the early units to be present. If this proves to be the case, then the whole evolution of the kidney of *R. temporaria* apparently took place along the following lines:

An ancestral form, with a long larval life and consequently no need to produce an adult kidney in the quickest possible manner, has given rise firstly to the Urodelan kidney with its fairly rapid method of production (the budding off of later units

from a functional early unit); and secondly, the kidney just described, in which the period occupied by Urodeles in the formation of a nephrically functional collecting trunk has been suppressed, and further, in which a method of producing a number of malpighian units in quick succession has been evolved.

So much for the malpighian units; now let us examine the question of the peritoneal funnels. In Urodeles the funnels lead from the coelom into a short tubule which opens directly into the cavity of a malpighian capsule—that is to say, the coelomic fluid is in osmotic connexion with the blood-supply. For some reason—the exact reason I leave to the physiologist—*Rana* has found it necessary to have a direct connexion between the coelomic fluid and the blood-supply at the earliest possible moment. This need has been met by the early units, in which those cells which would have formed the tube leading from the funnel to the capsule remain quiescent and the funnel itself is therefore forced to open into the blood-supply. Now, not only does it appear to be necessary that the funnels should be formed as early as possible, but also that they should continue to be formed as rapidly as possible; but the method adopted for the early production of malpighian capsules definitely cut down the rate at which funnels could be formed, for each malpighian capsule takes some time to reach the periphery of the kidney. It therefore became impossible for the production of the peritoneal funnels and malpighian capsules to remain in any way correlated with each other—hence the evolution of the funnel-forming tubule which carried the developing funnel directly to the point at which it was most required.

It would be as well to seize this moment to digress slightly and to demonstrate that no research is ever altogether new. Surprising though it may sound, this funnel-forming tubule was observed by Spengel, the earliest of all workers upon the Anuran kidney. He started with the assumption that the funnel must open into a capsule, but failed to find any proof of this; he did, however, find a single long tubule, internally ciliated at its tip, which he thought that he had succeeded in tracing into the collecting trunk. This error on his part is the more easy to

understand when we remember that both the collecting trunk and the funnel-forming tubule end blindly in the upper blastema mass ; it obviously requires only a very slight mistake to connect two tubules whose blind ends are separated at the most by a millimetre of irregular tissue.

So far we have offered no explanation as to the method by which these funnel-forming tubules might have been evolved ; for our suggestions let us turn once more to the Urodeles. Fürbringer, Hoffmann, and Farrington have all recorded that both the funnels and the connexion of the funnels to the capsules split in Urodeles so that two funnels may lead into a single capsule. If we postulate that this splitting process slowly receded farther and farther along the length of the unit, we obtain a condition in which the funnel and malpighian capsule form the two short arms of a ' Y ' ; this condition would be the less easy to visualize were it not described by Hall as a stage in the development of a unit of *Rana sylvatica*. From this stage to a ' V '—one of whose arms bears a developing malpighian capsule and the other a developing funnel—is but a short step, and from this to the condition in *R. temporaria* in which the two arms of the ' V ' develop entirely independently of each other, a still shorter step. I do not, of course, suggest that the funnel ever opened into the unit behind the malpighian capsule—but I do suggest that the funnel-forming tubule may have done so.

There is one further point in the earlier literature which bears out both the existence and the function of these funnel-forming tubules ; this point is that Fürbringer, Spengel, and Farrington have all commented upon the extraordinary manner in which the peritoneal funnels of the adult are grouped along the course of the blood-vessels. This ceases to be extraordinary when we remember that this is the very course followed by the funnel-forming tubule.

I trust, therefore, that I have succeeded in showing what I stated at the beginning of this discussion would be shown—that however extraordinary the course of the development of the mesonephros of *R. temporaria* may appear, it is

possible to show both how such a course of development may have been derived from an earlier form and to find corroborative evidence for it in the accounts of other workers.

In conclusion, I would like to record my great gratitude to Professor E. W. MacBride, F.R.S., who not only suggested that I should work upon this subject, but has also given me the greatest help and encouragement throughout the whole course of the research. Mr. H. R. Hewer, M.Sc., has also provided me with many helpful suggestions and has freely given me his assistance with the technical problems encountered.

I would also like to express my appreciation of the many constructive criticisms which have been offered by my friends and colleagues in the Zoological Research Laboratory of the Imperial College of Science, amongst whom Mr. H. K. Mookerjee and Miss D. E. Sladden have been especially helpful; indeed, it is to her skill in the rearing of animals under artificial conditions that I am indebted for the greater part of my material.

My friend Miss Kitty Edridge also afforded me considerable assistance in the somewhat tedious work of transferring the reconstruction in Text-fig. 1 from squared paper to its present form.

SUMMARY.

1. The entire mesonephros of *R. temporaria* is derived from the mesonephric blastema, a mass of cells originally occupying a position along the dorso-medial wall of the archinephric duct and later along the dorso-medial angle of the kidney.

2. The mesonephros arises as two perfectly distinct sets of units which are termed the 'early units' and the 'later units'.

3. The early malpighian units are derived as from small spherical condensations (termed 'nephroblast vesicles') in the blastema.

4. Each of these nephroblast vesicles elongates into a tubule which forms a connexion with the archinephric duct at one end and develops a malpighian capsule at the other.

5. These early units later sever their connexion with the archinephric duct and degenerate.

6. The early peritoneal funnels are derived from masses of blastema lying between the early malpighian capsules and the periphery of the mesonephros.

7. The lumina of these funnels never form any connexion with the lumina of the malpighian capsules, but form a direct connexion between the coelom and the blood-system.

8. The growth and coiling of the early units forces the archinephric duct away from the blastema into the dorso-lateral angle of the kidney.

9. During the course of this separation a special set of condensations arise.

10. These condensations elongate into straight tubules which maintain a connexion between the archinephric duct (into which they open) and the blastema.

11. That end of the straight tubule which lies in the blastema develops an abortive malpighian capsule at its tip.

12. The later malpighian units arise as condensations ('capsuloblast vesicles') in the blastema when this latter has become separated from the archinephric duct.

13. The capsuloblast vesicles are not formed singly but in vertically hanging strings; this is due to the fact that as each vesicle develops it is pushed downwards by the growth of the blastema above it, while a further vesicle condenses in the clear patch of blastema so left.

14. The capsuloblast vesicle differentiates into capsule and glomerulus, from the former of which a tubule grows out.

15. This tubule forms a connexion with a bud which has grown out from one of the straight tubules.

16. When the most ventral capsule of each string approaches the peritoneal wall of the kidney, it is seen to be separated from the latter by a small mass of blastema.

17. This mass of blastema develops into a peritoneal funnel exactly as do the blastema masses lying between the early malpighian capsules and the peritoneum.

18. A further set of condensations (the vesicles of the 'funnel-forming tubule') now arise in the blastema.

19. Each of these vesicles elongates into a tubule which runs down towards the peritoneal wall of the kidney.

20. The tubule so formed becomes internally ciliated along the lower third of its length.

21. The tip of one of these tubules enters one of the peripheral blood-vessels and places itself parallel to the outer wall of the mesonephros.

22. The whole tip of the tubule now breaks off and, by acquiring a connexion with the coelom, becomes a perfect peritoneal funnel.

23. It is suggested that the 'early units' represent an ancestral mesonephros and that the later units are homologous with the whole of the Urodelan mesonephros as it is at present known.

GLOSSARY OF NEW TERMS.

In view of the fact that several new terms have been coined to describe structures whose existence or function has hitherto been unknown, it has been thought as well to include a glossary of these new terms.

Capsuloblast vesicle.—A condensation in the blastema which gives rise to a later malpighian capsule and glomerulus.

Nephroblast vesicle.—A condensation in the blastema which gives rise to an entire early malpighian unit.

Funnel-forming tubule.—A tubule whose sole function is the production of later peritoneal funnels.

Straight tubule.—Employed as synonymous with, but more descriptive than, the earlier term 'collecting trunk'.

BIBLIOGRAPHY.

Only those titles actually referred to in the text are given; a very complete bibliography is to be found in the work of Hall.

Clarke (1881).—"The early development of the Wolffian body in *Amblystoma punctata*", 'Studies from the Biological Laboratory of Johns Hopkins University'; vol. 2.

- Farrington (1892).—"The nephrostomes of Rama", 'Transactions of the Connecticut Academy of Science', vol. 8.
- Filatow (1904).—"Entwicklungsgeschichte des Excretionsystems der Amphibien", 'Bulletin de la Société des Naturalistes de Moscou', vol. 18.
- Fürbringer (1887).—"Zur Entwicklung der Amphibieniere." (Dissertation.) Heidelberg.
- Goette (1875).—"Entwicklungsgeschichte der Unke." (Dissertation.) Leipzig.
- Hall (1901).—"The development of the mesonephros and Müllerian ducts in Amphibia", 'Bulletin of the Museum of Comparative Anatomy and Zoology of the University of Harvard', vol. 45.
- Hoffmann (1886).—"Zur Entwicklungsgeschichte der Urogenitalorgane bei den Anamnia", 'Zeitschrift für wissenschaftliche Zoologie', vol. 44.
- Marshall and Bles (1890).—"The development of the kidneys and fat bodies in the frog", 'Studies from the Biological Laboratory of Owens College', vol. 2.
- Nussbaum (1880).—"Endigung der Wimpertrichter i. d. Niere der Anura", 'Zoologischer Anzeiger', vol. 3.
- (1887).—"Geschlechtstheil der Froschniere", *ibid.*, vol. 20.
- Spengel (1876).—"Das Urogenitalsystem der Amphibia", 'Arbeiten vom Zoologischen Institut der Universität Württemberg', vol. 3.

EXPLANATION OF PLATES 27-31.

LIST OF COMMON ABBREVIATIONS.

a.d., archinephric duct; *a.m.c.*, abortive malpighian capsule; *b.*, blastema; *b.m.*, point of separation of funnel from funnel-forming tubule; *b.v.*, blood-vessel; *cp.v.*, capsuloblast vesicle; *d.m.c.*, degenerating malpighian capsule; *f.k.*, rudiment of malpighian tubule; *f.n.*, rudiment of peritoneal funnel; *f.st.*, rudiment of straight tubule; *i.n.*, opening of peritoneal funnel to blood-vessel; *k.*, malpighian tubule; *m.*, myotome; *m.c.*, malpighian capsule; *n.*, peritoneal funnel; *nst.t.*, funnel-forming tubule; *nst.v.*, vesicular rudiment of funnel-forming tubule; *o.n.*, opening of peritoneal funnel from coelom; *o.st.*, outgrowth from straight tubule; *r.p.t.*, retro-peritoneal connective tissue; *st.*, straight tubule.

PLATE 27.

Figs. 1 to 6.—Transverse sections across the nephric region of a 17 mm. tadpole, in the regions indicated in Text-fig. 1.

PLATE 28.

Figs. 7 and 8.—Microphotographs of sections of the mesonephros of a 20 mm. tadpole.

Fig. 7. Transverse section.

Fig. 8. Obliquely parasagittal section.

PLATE 29.

Fig. 9.—Reconstruction of typical units from the mesonephros of a 20 mm. tadpole.

Black, main outline of mesonephros; red, peripheral blood-system; blue, archinephric duct and straight tubule; yellow, malpighian capsule and its attendant tubule; green, peritoneal funnel and funnel-forming tubule.

PLATE 30.

Figs. 10 to 14.—Sections to illustrate the development of the capsuloblast vesicles.

Figs. 10 and 12.—T.S. mesonephros of newly metamorphosed frog.

Figs. 11, 13, and 14.—T.S. mesonephros of a 20 mm. tadpole.

PLATE 31.

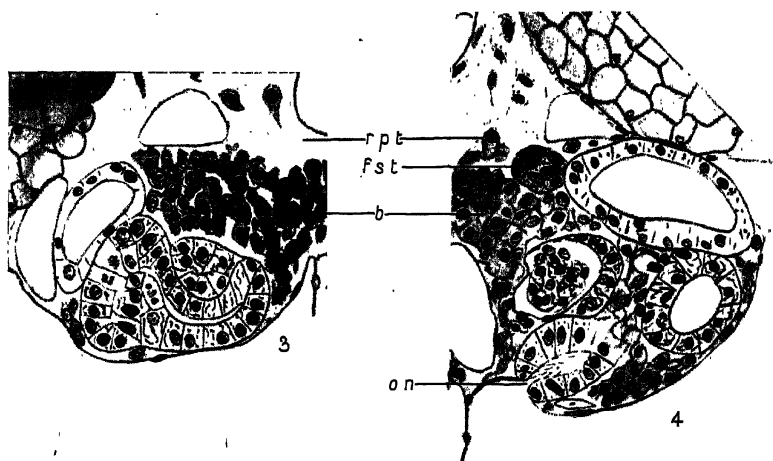
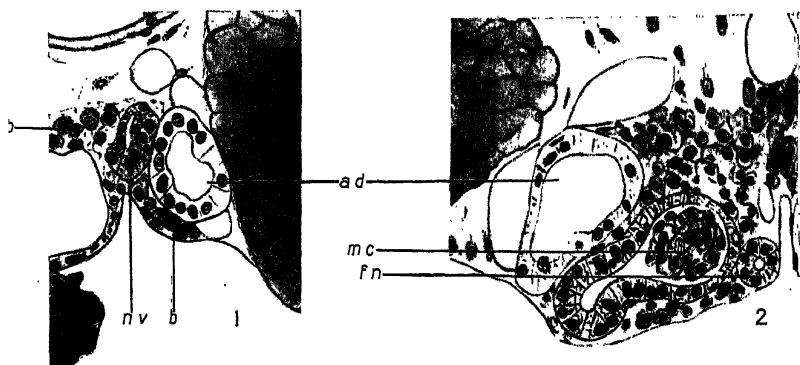
Figs. 15 to 22.—Sections illustrating the development of the straight tubule and of the later peritoneal funnels.

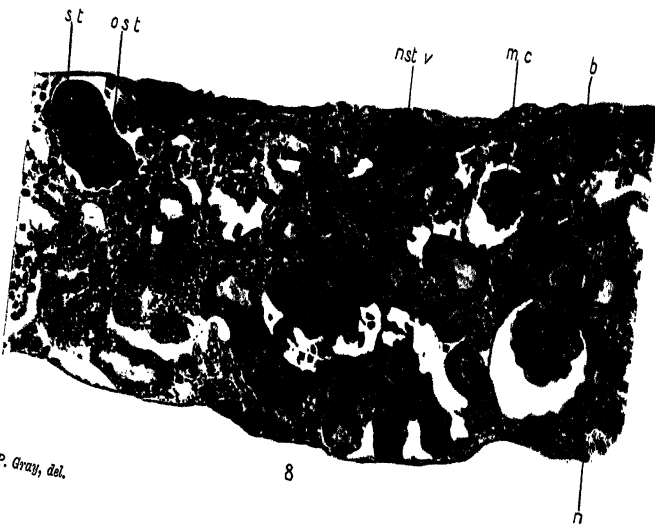
Fig. 15.—T.S. mesonephros of a 24 mm. tadpole.

Figs. 16–19.—T.S. mesonephros of a 30 mm. tadpole.

Figs. 20–2.—T.S. mesonephros of a 20 mm. tadpole. These three sections are taken at 25μ intervals from a regular series.

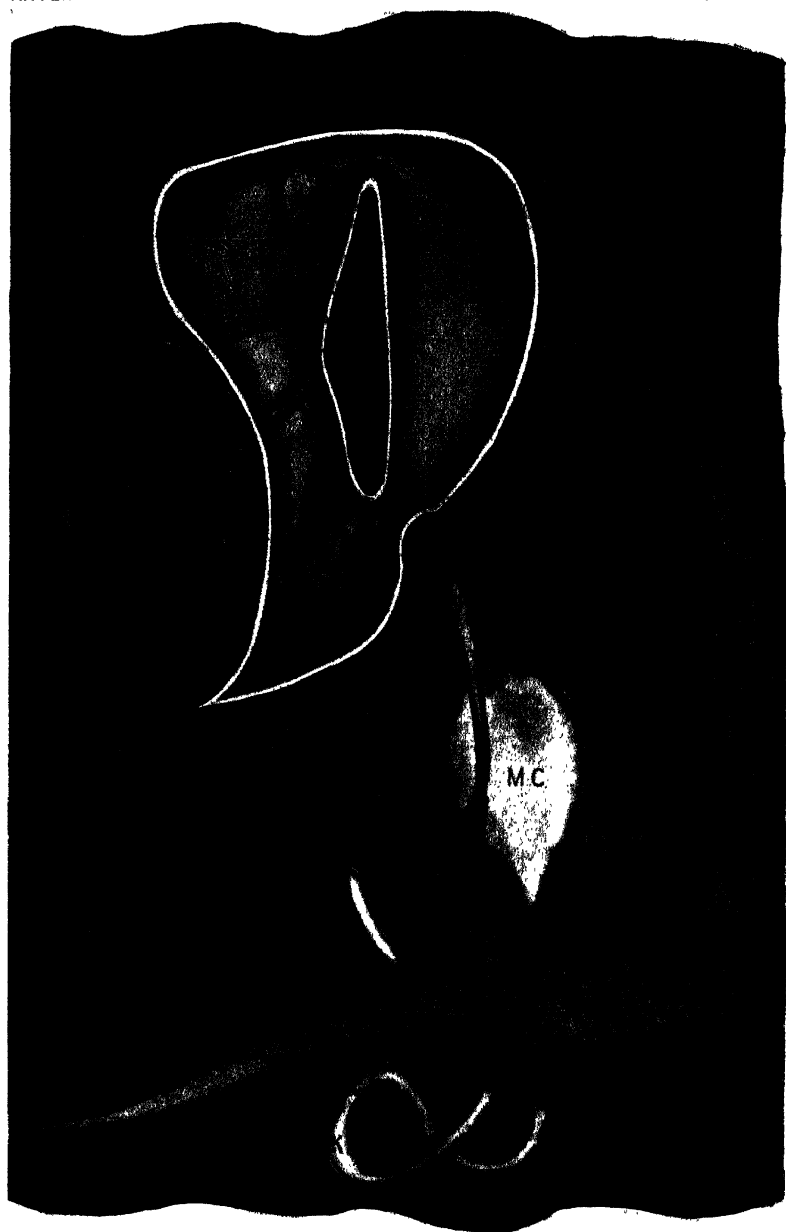
Unless otherwise stated, all figures are from camera lucida drawings. For explanation of exact stage denoted by tadpole length, see table on p. 511.





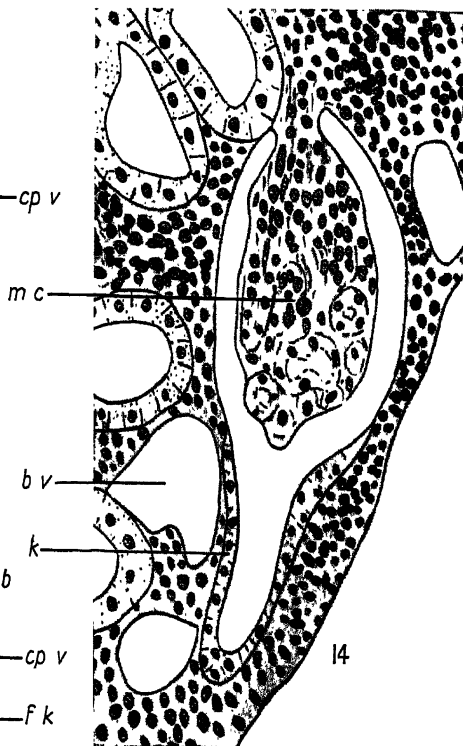
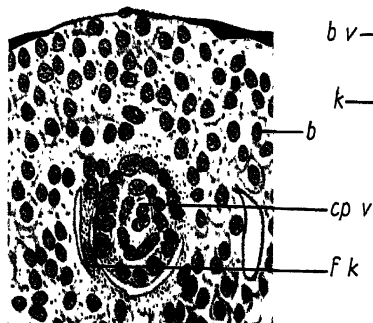
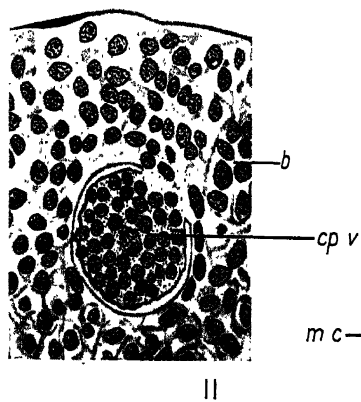
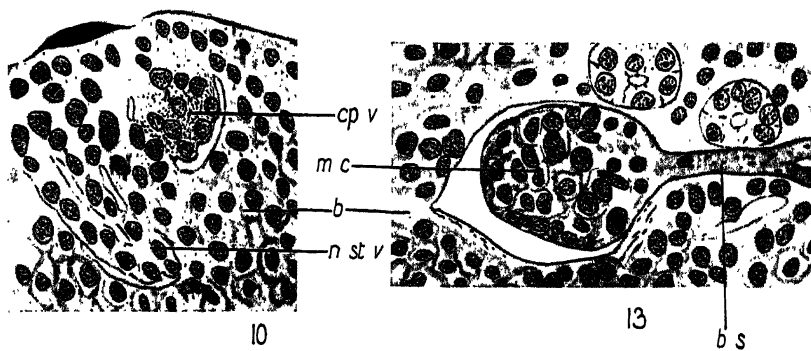
ANTERIOR

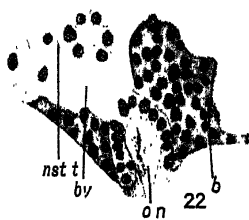
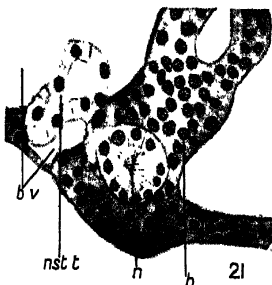
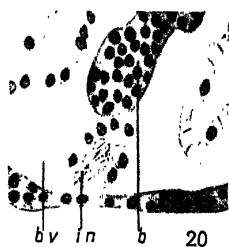
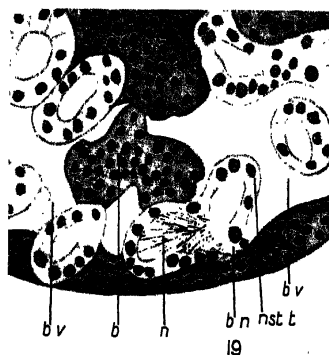
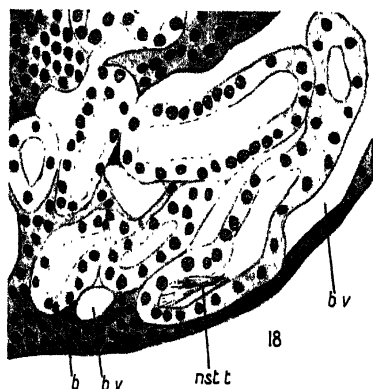
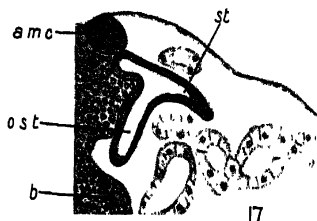
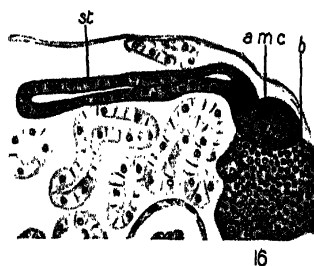
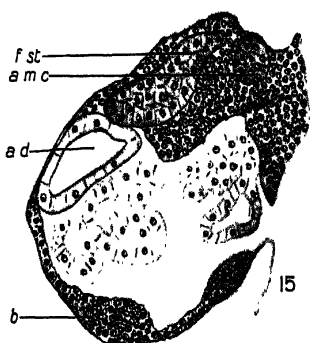
POSTERIOR



ANTERIOR

POSTERIOR







**Le Cycle chromosomique d'une nouvelle
Actinomyxidie: *Guyénotia sphaerulosa*
N. gen.; n.sp.**

par

André Naville,

Chef des Travaux de Zoologie à la Faculté des Sciences de Genève.

Avec planches 32 à 34, et trois figures dans le texte.

SOMMAIRE.

	PAGES
INTRODUCTION	547
POSITION SYSTÉMATIQUE DE LA FORME ÉTUDIÉE	548
TECHNIQUE	550
PREMIÈRES PHASES DU DÉVELOPPEMENT DU SPOROZOÏTE JUSQU'À LA CONSTITUTION D'UN CYSTE À DEUX CELLULES INTERNES	551
ÉVOLUTION DES CELLULES INTERNES: GAMÉTOGÈNESE	554
LE SYNCHRONISME AU COURS DES DIVISIONS DU PANSPOROOCYSTE	561
LA RÉDUCTION CHROMATIQUE DES ACTINOMYXIDIES	562
GRANULES CHROMATOÏDES DU PANSPOROOCYSTE	563
LA FÉCONDATION	564
LES DIVISIONS DU ZYGOTE	564
FORMATION DE L'ÉBAUCHE SPORALE, PUIS DE LA SPORE	565
CONCLUSIONS	570
INDEX BIBLIOGRAPHIQUE	572
EXPLICATION DES PLANCHES	573

INTRODUCTION.

Mes recherches sur la sexualité chez les Myxosporidies m'ont conduit à décrire les phénomènes réductionnels chez ces Sporozoaires.

L'étude de la gamétogénèse me révéla, d'autre part, l'existence de plusieurs types d'anisogamie qui semblaient déterminés par le moment précis où la sexualisation des éléments germinaux devenait observable. Cette sexualisation peut, en effet, se produire tardivement, après les phénomènes réductionnels (cas de *Myxidium incurvatum* Thélohan), ou au contraire

apparaître déjà avant la réduction chromatique (cas de *Sphaeromyxa*). Dans un autre type intermédiaire (*Myxobolus guyénoti* Naville) on s'aperçoit que c'est au cours de la deuxième cinèse réductionnelle que la 'polarité sexuelle' du Cyte II se manifeste par une plasmotomie hétéropolaire.

Je désirais étudier en détail les phénomènes réductionnels chez une *Actinomyxidie*, et vérifier si, comme je le pensais, la sexualisation apparaissait chez ces animaux déjà au moment des 'cinèses goniales'. Cette sexualisation très précoce m'apparaissait comme un mode de sexualité transitoire conduisant à la dioecie.

J'ai eu la chance de trouver cet été des *Actinomyxidies* d'un genre nouveau : *Guyénotia sphaerulosa*, en très grande abondance. Depuis plusieurs années déjà je cherchais des *Actinomyxidies*, mais sans succès. Je suis particulièrement heureux de pouvoir dédier cette nouvelle forme à Monsieur le Professeur E. Guyénot.

POSITION SYSTÉMATIQUE DE LA FORME ÉTUDIÉE.

A la fin de son excellente étude sur les *Actinomyxidies* Granata a dressé un tableau systématique des genres et des espèces connus jusqu'à ce jour. Cet auteur divise les *Actinomyxidies* en deux familles :

1^o Les *Haploactinomyxidae* dont le seul genre *Tetractinomyxon* Ikeda est caractérisé par la structure sporale : Involucre sporal formé d'une épispore tricellulaire et d'une endospore monocellulaire. Chaque spore ne contient qu'une seule cellule propagative binucléée. L'espèce décrite par Ikeda (*Tetractinomyxon intermedium*) est parasite d'un Sipunculier, le *Petalostoma minutum* Kef.

2^o Les *Euactinomyxidae* qui présentent un involucre sporal formé de trois cellules valvaires, pouvant, dans certains genres, former des expansions ou appendices caractéristiques des genres. La masse germinale de la spore est multinucléée et se résout en cellules propagatives mononucléées. Cette famille comporte jusqu'à ce jour cinq genres que Granata caractérise à l'aide de la clef suivante :

- I. Cellules de la paroi sporale (cellules valvaires) tendant à former une simple enveloppe membraneuse sans prolongements.

Genre *Sphaeractinomyxon* Caull. et Mesn.

- II. Cellules de la paroi sporale (cellules valvaires) présentant des prolongements :

- A. Chaque spore présente trois prolongements aliformes :

- (a) Spore en forme d'ancre à trois bras.

Genre *Triactinomyxon* Stolc.

- (b) Spore en forme d'ancre à six bras.

Genre *Hexactinomyxon* Stolc.

- B. Deux prolongements aliformes allongés et un appendice beaucoup plus court.

Genre *Synactinomyxon* Stolc.

- III. Cellules de la paroi sporale (cellules valvaires) globuleuses, formant trois petits appendices sphaéroïde.

Genre *Neactinomyxon* Granata.

Comme on le voit, par ce tableau, les genres actuels d'Actinomyxidiées sont extrêmement faciles à caractériser et la systématique de ce groupe présente, de ce fait, peu de difficultés.

La forme d'Actinomyxidie dont l'étude fait l'objet de ce mémoire ne rentre dans aucun des genres décrits jusqu'à ce jour. Je l'ai rencontrée en Août 1929 chez des *Tubifex tubifex* Müll. recueillis à Luc-sur-Mer (Calvados) dans un petit ruisseau à fond vaseux. Cette nouvelle forme est parasite de l'épithélium intestinal de l'Oligochète qu'il envahit parfois complètement. Les spores, au nombre de huit dans chaque pansporocyste, sont globuleuses, presque sphériques, mesurant 15μ de diamètre. Leur extrémité antérieure montre, vue de face, trois lignes suturales qui convergent au pôle antérieur et forment entre elles un angle d'environ 120° . Le pôle antérieur porte trois capsules polaires pyriformes, presque sphériques, de 6μ de long sur 5μ de large. Chaque capsule polaire vient déboucher isolément sur une des lignes suturales à $2\frac{1}{2}\mu$ du pôle antérieur de la spore, point de jonction des trois sutures. À la partie postérieure de la

spore, on observe trois grands appendices digitiformes contenant, au niveau de leur tiers postérieur, un noyau chacun. Ces trois appendices sont égaux, ce qui permet de distinguer facilement la spore de cette forme nouvelle des spores du genre *Synactinomyxon* de Stoll.

Je propose pour cette nouvelle forme le nom de *Guyénotia sphaerulosa* que j'ai le plaisir de dédier à Monsieur le Professeur E. Guyénot en témoignage d'affection et d'estime.

Le tableau dichotomique de Granata pourra donc être complété par l'introduction du genre *Guyénotia* en II A (c).

Diagnose de *Guyénotia sphaerulosa*. g. et sp. nov.

Spores groupées par huit dans un pansporocyste à quatre noyaux. Spore sphaéroïdale présentant trois lignes suturales à 120°, trois capsules polaires débouchant sur chacune des trois lignes suturales à une petite distance de l'apex de la spore. La spore porte trois appendices digitiformes nucléés égaux et plus longs que le diamètre de la spore. Sporoplasme multinucléé à trente-deux noyaux. Diamètre de la spore : 15 μ . Longueur des appendices d'une spore mûre : 40 μ .

Parasite de l'épithélium intestinal de *Tubifex tubifex* Müll. ; habitat : Luc-sur-Mer (Calvados).

Cette espèce parasite les cellules de l'épithélium intestinal de l'Oligochète, où se produit d'ailleurs tout le développement. Il arrive parfois que les parasites envahissent non seulement la sous-muqueuse, mais encore le tissu chloragogène et même le coelome. Mais ce cas est exceptionnel. Il semble dû à un parasitisme trop intensif et conduit presque toujours à des pansporocystes dégénérés.

TECHNIQUE.

Les *Tubifex tubifex* provenant de Luc-sur-Mer,¹ et que j'ai pu avoir en très grande abondance, se sont montrés très fortement parasités. Un exemplaire sur deux ou trois présentait

¹ Je tiens à remercier ici Monsieur le Professeur L. Mercier, qui, grâce à sa si gentille hospitalité, m'a permis de trouver ce matériel ; et Madame Le Roux qui a eu l'obligeance de m'expédier, par deux fois, des *Tubifex* de Luc-sur-mer.

une forte infection. Il m'a donc été très facile d'obtenir un matériel abondant. Après avoir utilisé les fixateurs les plus divers, tels que le liquide de Schaudinn, le sublimé acétique, le mélange de Zenker, le liquide de Bouin sublimé, le liquide de Bouin modifié par Hollande, le mélange alcoolique de Duboscq et Brasil, le liquide de Flemming, le mélange de Champy ainsi que le liquide de Regaud IV suivi d'une post-chromisation, je me suis arrêté aux deux mélanges qui m'ont incontestablement donné les meilleurs résultats. Ces deux fixateurs sont : le liquide de Duboscq et Brasil et le mélange de Bouin-Hollande. Les pièces fixées par ce dernier procédé sont ensuite lavées au liquide de Bouin pour éliminer l'acétate de cuivre, puis transportées dans l'alcool à 50° puis à 70°.

Pour l'emparaffinage j'ai utilisé les procédés habituels, en passant par l'essence de girofle (24 heures), puis par un bain de quelques minutes dans le Xylol. Les coupes faites presque toutes à 8 μ d'épaisseur ou plus, ont été colorées à l'hématoxyline de Heidenhain, suivant le procédé usuel. Cependant quelques unes d'entre elles ont été différenciées non pas à l'alun de fer, mais à l'alcool acide. Ce procédé permet d'obtenir une différenciation plus égale qui est précieuse pour l'examen des spores et pour les dernières phases de l'évolution sporale. J'ai également étudié l'évolution de la spore sur frottis humides colorés au Giemsa.

Tous les dessins ont été exécutés à la Chambre claire, en utilisant un objectif à immersion de 1/12ème de Leitz et un oculaire périplan $\times 25$ de la même marque.

PREMIÈRES PHASES DU DÉVELOPPEMENT DU SPOROZOÏTE JUSQU'À LA CONSTITUTION D'UN CYSTE À DEUX CELLULES INTERNES.

Lorsqu'une spore d'Actinomyxidie est mûre, elle contient, à l'intérieur de l'endospore, un nombre variable de sporozoïtes. Le nombre de ces sporozoïtes semble constant pour chaque espèce. Suivant les espèces considérées, les sporozoïtes — qui représentent exactement le germe ou sporoplasme des autres Néosporidies — possèdent un ou deux noyaux. Ce sont ces

petits corpuscules qui constituent l'élément propagateur du parasite. L'évolution de ces sporozoïtes conduit à la formation de petits kystes à l'intérieur desquels se forment huit spores. Les tout premiers stades de l'évolution du sporozoïte, à l'intérieur des tissus de l'hôte, présentent une certaine difficulté d'observation et divers points, relatifs à cette première période de l'évolution du parasite, sont encore restés dans l'ombre.

Chez le *Tetractinomyxon intermedium*, Ikeda montre que le sporozoïte unique de la spore est toujours binucléé. Dans les tissus de l'hôte il se rencontre également toujours sous la forme d'un petit élément binucléé dont les deux noyaux sont intimement accolés. Le sporozoïte possède donc toujours deux noyaux, puis forme un groupe de quatre éléments.

Chez *Sphaeractinomyxon stolci*, Caullery et Mesnil ont montré que la spore contient un grand nombre d'éléments mononucléés. Au début de l'infection, ces auteurs observent de nombreuses formes binucléées. L'origine de ces formes binucléées reste incertaine. Caullery et Mesnil tiennent pour vraisemblable que la forme binucléée provient de la plasmogamie de deux sporozoïtes mononucléés. Ils considèrent cependant l'hypothèse inverse comme possible : soit la division du noyau du sporozoïte, formant ainsi un corps binucléé.

Chez *Sphaeractinomyxon gigas*, Granata observe, dans le tissu chloragogène, des accumulations grégaires de corpuscules binucléés. Il est, par suite, conduit à admettre l'existence d'une multiplication agame des éléments binucléés. Cette hypothèse semble confirmée par le fait que Granata observe assez fréquemment chez le *Triactinomyxon gigas* de jeunes kystes jumeaux qui résulteraient de la scissiparité d'éléments binucléés. Granata pense donc que l'état binucléé précède l'état mononucléé.

Léger, étudiant les premières phases du *Triactinomyxon*, fut conduit à admettre l'existence d'une copulation de deux sporozoïtes, dont les noyaux, tout d'abord indépendants, finissent par fusionner. Enfin, Mackinnon et Adam, qui ont examiné en détail le cycle de trois espèces de *Triactinomyxon*, montrent que la plasmogamie de deux sporozoïtes

forment un corps binucléé, origine du groupe de quatre cellules, pour autant que les corps binucléés ne sont pas observables déjà à l'intérieur de la spore (*Triactinomyxon légeri*).

Mes observations personnelles concernant les premières phases du développement de *Guyénotia sphaerulosa* me conduisent à des conclusions analogues à celles de Mackinnon et Adam. Ayant examiné avec soin un très grand matériel de coupes, je n'ai jamais rencontré de sporozoïtes isolés, ni de formes mononucléées. Il est donc vraisemblable que l'appariement des sporozoïtes se fait immédiatement, dès l'éclosion de la spore. D'autre part, les éléments binucléés de taille variable étaient parfois si abondants chez certains individus de *Tubifex* que les phénomènes de cytodierèse, aboutissant à la formation de corps mononucléés, n'auraient guère pu m'échapper. Je crois donc que l'interprétation de Mackinnon et Adam s'applique très exactement au cycle de *Guyénotia sphaerulosa*.

Les corps binucléés les plus petits observés ne dépassent guère 4μ sur 5μ , les noyaux présentent un diamètre de 2μ . La figure 1 (Pl. 32) montre que les noyaux sont intimement pressés les uns contre les autres. Leur zone de contact se montre particulièrement sidérophile et forme une sorte de barre transversale noirâtre, dans les préparations colorées à l'hématoxyline. Ces corps binucléés s'accroissent sensiblement jusqu'à atteindre une taille de 9 à 10μ de longueur. Les noyaux sont toujours au contact l'un de l'autre, mais leur accollement est moins intime (fig. 2, 3, et 4, Pl. 32). C'est à ce moment que les noyaux entrent en prophase cinétique et laissent apercevoir un fort spirème (fig. 5, Pl. 32). L'apparition des phénomènes mitotiques n'est d'ailleurs pas toujours synchrone dans les deux noyaux du corps binucléé et il arrive parfois qu'une des deux cinèses est déjà complètement achevée alors que le noyau retardataire montre les premiers aspects prophasiques (fig. 6, Pl. 32). Lorsque les deux cinèses sont achevées, on observe alors un corps à quatre noyaux (fig. 7, Pl. 32), dont deux sont sensiblement plus petits. C'est à ce moment seulement que la plasmodomie se manifeste et il se forme deux grandes cellules centrales entourées par deux cellules pariétales qui enveloppent les

premières. Les cellules pariétales formeront les éléments de la paroi du pansporocyste (fig. 8, Pl. 32). Les noyaux des cellules pariétales ne tarderont pas à se diviser (fig. 9, Pl. 32). La paroi du pansporocyste comprendra alors quatre noyaux qui ne se diviseront plus.

Comme on le voit, les premières phases du développement de *Guyénotia sphaerulosa* sont tout à fait conformes au schéma classique. La plasmotomie est cependant plus tardive que chez le *Sphaeractinomyxon gigas* de Granata. Mes observations concernant cette première période du cycle évolutif de *Guyénotia* sont tout à fait superposables aux faits décrits par Mackinnon et Adam.

Durant toute cette première période du cycle, on observe fréquemment des cinèses ; malheureusement, le tassement de la substance chromatique est tel qu'elles ne se prêtent pas à des numérations chromosomiques certaines. Il semble cependant certain qu'elles sont toutes du type diploïde.

ÉVOLUTION DES CELLULES INTERNES : GAMÉTOGÈSE.

Les divisions successives des deux cellules internes de chaque pansporocyste aboutissent à la formation de seize éléments gamétiques. De ces seize éléments, huit sont plus gros et constituent les macrogamètes, tandis que les huit autres, de taille plus restreinte, sont les microgamètes. Caullery et Mesnil, dans leur excellent travail sur *Sphaeractinomyxon stolci*, ont décrit très exactement toutes les phases de ce développement. Par la suite, Granata, puis Mackinnon et Adam n'ont pu que vérifier leurs observations dans des genres voisins. Au cours de la formation des gamètes, on peut observer six phases successives dans l'évolution de la lignée germinative, phases qui sont dues au fait que les éléments de la lignée mâle et de la lignée femelle ne se divisent jamais en même temps ; les éléments femelles manifestent durant toute la gamétogénèse un retard très marqué, une inertie cinétique, par rapport à ceux de la lignée mâle. Cette différence dans la rapidité des divisions successives des éléments cellulaires s'explique fort bien, si l'on

pense que les éléments de la lignée mâle, restent sensiblement plus petits que ceux de la lignée femelle qui ne cessent de s'accroître.

D'après Caullery et Mesnil, puis Granata, des deux cellules internes du jeune pansporocyste, l'une d'entre elles se divise d'abord (cellule α), tandis que sa conjointe ne se divise que plus tard (cellule β). Il en résulte la formation d'un groupe de trois cellules : $\alpha_1, \alpha_2, \beta$.

La cellule β (élément gonial femelle) se divisant à son tour, on observe alors quatre éléments : $\alpha_1, \alpha_2, \beta_1, \beta_2$.

Les cellules α_1 et α_2 se divisant toutes deux, on obtient alors six éléments en tout : $\alpha_{11}, \alpha_{12}, \alpha_{21}, \alpha_{22}, \beta_1$, et β_2 .

La division suivante se produisant toujours dans la lignée mâle, on observe alors dix éléments : $\alpha_{111}, \alpha_{112}, \alpha_{121}, \alpha_{122}, \alpha_{211}, \alpha_{212}, \alpha_{221}, \alpha_{222}, \beta_1, \beta_2$.

Les cellules de la lignée femelle β_1 et β_2 , sortant de leur inertie cinétique, forment alors quatre éléments, ce qui porte le nombre total à douze : $\alpha_{111}, \alpha_{112}, \alpha_{121}, \alpha_{122}, \alpha_{211}, \alpha_{212}, \alpha_{221}, \alpha_{222}, \beta_{11}, \beta_{12}, \beta_{21}, \beta_{22}$.

Enfin, les quatre éléments β se divisant encore une fois, on observe alors seize éléments gamétiques : $\alpha_{111}, \alpha_{112}, \alpha_{121}, \alpha_{122}, \alpha_{211}, \alpha_{212}, \alpha_{221}, \alpha_{222}, \beta_{111}, \beta_{112}, \beta_{121}, \beta_{122}, \beta_{211}, \beta_{212}, \beta_{221}, \beta_{222}$.

Ces données ont été très bien établies par les cinq auteurs précités et mes observations personnelles n'ont pu que les confirmer.

Au cours des recherches qui ont été faites sur la gamétogenèse des Actinomyxidies, aucun auteur n'a étudié les figures cinétiques au point de vue chromosomique. Cette lacune est d'autant plus regrettable que l'étude chromosomique est la clef des phénomènes réductionnels si nécessaires pour la compréhension du cycle même des Néosporidies. Mes recherches, ayant pour objet principal l'étude des phénomènes réductionnels, c'est à l'examen des cinèses durant cette partie du cycle que je me suis principalement intéressé. Il y a six éclosions cinétiques successives jusqu'à la formation des gamètes. Je décrirai donc, avec quelques détails, ces six périodes cinétiques de la gamétogenèse. La figure schématique I fera facilement comprendre la succession des divisions cellulaires.

Première cinèse.

Le pansporocyste étant constitué (et présentant quatre cellules de la paroi et deux cellules internes), l'une de celles-ci, initiale de la lignée germinale mâle, entre en cinèse. Le spirème se décompose en éléments chromatiques qui, au moment de la dissolution de la membrane nucléaire, se trouvent déjà au nombre de huit (fig. 11, Pl. 32). Ceci nous montre que, comme on l'observe d'ailleurs, le spirème est en réalité un dispirème et chaque chromosome est, à ce moment, divisé en deux éléments. La fig. 11 montre deux groupes de quatre chromosomes, qui gagneront chacun des deux pôles cellulaires lorsque la figure astérienne fera son apparition. La fig. 13 (Pl. 32) montre l'achèvement de cette première division et la prophase de la division suivante. Le pansporocyste contient alors trois éléments de quatre chromosomes chacun, soit le nombre diploïde. On peut donc considérer cette première cinèse, qui donne naissance aux éléments α_1 et α_2 , comme une cinèse goniale de la lignée mâle.

Deuxième cinèse.

La première cinèse n'est point encore achevée et les deux éléments α_1 et α_2 ne sont point encore au repos que le noyau de la cellule conjointe β , origine de la lignée germinale femelle, entre également en prophase. Les processus cinétiques reproduisent exactement les principales phases observées au cours de la première cinèse (lignée mâle). Sitôt la membrane nucléaire dissoute et le dispirème rompu, les huit éléments chromatiques apparaissent très nettement (fig. 13, Pl. 32). Ils se répartiront, par la suite, en deux groupes de quatre éléments chacun et gagneront ainsi les deux pôles cinétiques, lorsque la figure achromatique et les deux centres seront apparus.

La fig. 14 (Pl. 32) nous montre cette migration polaire. Elle indique, en outre, qu'après la phase de condensation télophasique des deux noyaux α_1 et α_2 , survient une période de courte durée, pendant laquelle les chromosomes—tout d'abord fortement comprimés aux deux pôles cinétiques et, de ce fait, indiscernables—réapparaissent au nombre de quatre, immédiate-

ment avant la période de repos des noyaux. Cette seconde cinèse de la lignée femelle donne naissance aux éléments β_1 et β_2 . Le pansporocyste contient alors quatre cellules internes et deux (fig. 10, Pl. 32) ou quatre cellules de la paroi¹ (fig. 12, Pl. 32).

Cette seconde cinèse correspond donc à une cinèse goniale de la lignée femelle. Le noyau se montre à l'état diploïde et les éléments qui naissent ainsi sont chacun pourvus de quatre chromosomes. Jusqu'ici donc le parallélisme est complet entre l'évolution de la lignée mâle et de la lignée femelle. Seul le retard manifesté chez cette dernière permet de distinguer les deux sexes. Les quatre cellules des pansporocystes présentent une taille presque identique et il est souvent difficile de distinguer les éléments mâles des éléments femelles. Chez d'autres espèces, telles que le *Triactinomyxon légeri*, d'après Mackinnon et Adam, cette différence de taille est nettement plus marquée. Ce n'est que plus tard que la différence de taille des éléments s'accentuera.

Troisième cinèse.

La troisième cinèse, comme le montre le schéma (voir fig. 1), intéresse de nouveau les éléments de la lignée mâle. Elle se produit après une assez longue période de repos, durant laquelle le pansporocyste ne présente que quatre cellules internes. L'abondance des éléments à quatre cellules nous indique clairement que cette période doit être de quelque durée. Les deux 'gonies' de la lignée mâle α_1 , et α_2 se transforment alors en véritables cytes de premier ordre. La figure diakinétique montre l'apparition de quatre chromosomes, mais je n'ai pu, dans aucun cas, préciser (dans la lignée mâle tout au moins, où la succession des diverses phases semble plus précipitée) s'il existe un groupement par paires (diploténie). Ces chromosomes, sitôt la membrane nucléaire disparue, se mettent

¹ La cinèse qui divise les deux éléments primitifs de la paroi du pansporocyste, peut se produire plus ou moins tôt. C'est la raison pour laquelle on rencontre parfois des pansporocystes à quatre cellules internes, mais ne possédant encore que deux cellules de la paroi.

au fuseau et forment, en vue polaire, de petits groupements de quatre chromosomes, tout à fait semblables à ceux que l'on observe sur la fig. 14 (Pl. 32).¹ Le nucléole gagne habituellement un des pôles de la cinèse, ou bien se trouve provisoirement rejeté dans le cytoplasme. Les chromosomes ne se divisent pas, mais deux d'entre eux sont attirés vers l'un des pôles, alors que les deux autres ainsi que le nucléole gagnent le centre cinétique opposé. La fig. 18 (Pl. 32) montre une anaphase de cette troisième cinèse. Comme l'indiquent les deux cellules de droite de la fig. 14, on remarque que la garniture chromosomique à l'état diploïde (quatre chromosomes) contient deux éléments plus grands que les deux autres. Ces deux grands éléments sont fréquemment arqués, tandis que les deux autres sont en bâtonnets. L'anaphase de la troisième cinèse, représentée à la fig. 18 (Pl. 32), montre que les deux constituants de la grande paire de chromosomes gagnent chacun l'un des pôles de la cinèse, de même que les constituants de la petite paire.

Cette troisième cinèse de la lignée mâle donne naissance aux cellules α_{11} , α_{12} , α_{21} , α_{22} . Avec les deux cellules β_1 et β_2 de la lignée femelle, on compte en tout six éléments. La division que l'on observe durant cette période est donc une division réductionnelle et hétérotypique, qui ramène le nombre des chromosomes de chaque noyau de quatre (nombre diploïde) à deux (nombre haploïde). Après la cinèse goniale (première cinèse), les cellules α_1 et α_2 passent à l'état de cyte I, et les éléments α_{11} , α_{12} , α_{21} , α_{22} correspondent alors aux cytes II.

Je n'ai pu déterminer s'il existe des phases préméiotiques. Le noyau, à l'approche de la cinèse, subit une sorte de tassement chromatique que l'on retrouve d'ailleurs très fréquemment dans les noyaux qui viennent de se diviser, ou qui, au contraire, vont

¹ Je n'ai pas pu décider si les deux noyaux de droite de la fig. 14 représentent des états post-télophasiques de la première cinèse (cellules α_1 et α_2) ou des états prophasiques (diakinèse) de la troisième cinèse (cellules β_1 et β_2). Cette seconde hypothèse me semble cytologiquement plus vraisemblable. D'autres figures de prophases réductionnelles sont identiques. Il faudrait, dans ce cas, admettre qu'il peut se produire accidentellement une accélération du développement et que la phase à quatre cellules quiescentes est alors inobservable.

entrer en cinèse (voir figs. 16, 20, et 21 de la Pl. 32). Il en résulte qu'un phénomène de synapsis peut passer inaperçu, étant donné sa grande analogie avec certains états post ou précinétiques.

Quatrième cinèse.

Si nous nous reportons au schéma de la fig. 1, nous voyons que la dernière division de la lignée mâle (éléments α_{11} , α_{12} , α_{21} , α_{22}) se produit avant le déclenchement de la cinèse réductionnelle de la lignée femelle. Les phénomènes prophasiques apparaissent donc dans les quatre cytes II de la lignée mâle lorsque le pansporocyste présente six éléments.

On voit apparaître, alors, tout de suite après la dissolution de la membrane nucléaire, deux petits éléments chromatiques (fig. 22, Pl. 33), disposés côte à côte au centre de la cellule dans une aire cytoplasmique plus claire. Lorsque la figure astérienne est apparue, ces éléments se divisent et chaque chromosome fils gagne l'un des pôles. Ces figures montrant deux chromosomes à chaque extrémité du fuseau sont extrêmement fréquentes, et une observation des coupes, même superficielle, permet à coup sûr d'en rencontrer. Elles sont identiques aux figures anaphasiques de la première cinèse réductionnelle, telle qu'on peut l'observer dans la fig. 18.

Cette dernière division, qui se montre homéotypique, aboutit à la formation des huit éléments gamétiques mâles.

L'évolution de la lignée germinale mâle est achevée et la multiplication des cellules germinatives femelles commence seulement.

Cinquième cinèse.

De même que la troisième cinèse du pansporocyste est la cinèse réductionnelle de la lignée mâle, la cinquième cinèse constitue la division hétérotypique de la lignée femelle. Cette phase fait passer du pansporocyste à dix cellules au pansporocyste à douze éléments. La fig. 21 (Pl. 32) montre les deux éléments β_1 et β_2 en prophase à côté de huit éléments gamétiques

mâles. Mais il arrive parfois que le synchronisme des deux cinèses réductionnelles de la lignée mâle n'étant pas respecté, la cinquième cinèse se produise déjà avant que les quatre éléments: α_{11} , α_{12} , α_{21} , α_{22} , soient entièrement divisés. La fig. 15 (Pl. 32) montre un cas semblable où l'un des cytes II de la lignée mâle reste de plus grande taille et n'est point encore divisé. Cette même fig. 15 nous montre deux aspects de la cinèse réductionnelle femelle. En haut de la figure, à droite, l'un des deux cytes I est en prophase, montrant quatre chromosomes groupés en deux paires et dont les constituants sont presque entièrement dissociés. Les deux chromosomes d'une même paire sont cependant encore au contact l'un de l'autre par l'une de leurs extrémités. Ce détail semble montrer que les constituants de chaque paire se trouvaient auparavant accouplés sur toute leur longueur. Ce seul fait semble indiquer un état prêmeiotique du noyau. La même fig. 15 montre, en outre, une anaphase très nette. Chaque pôle cinétique ne comporte alors que deux chromosomes.

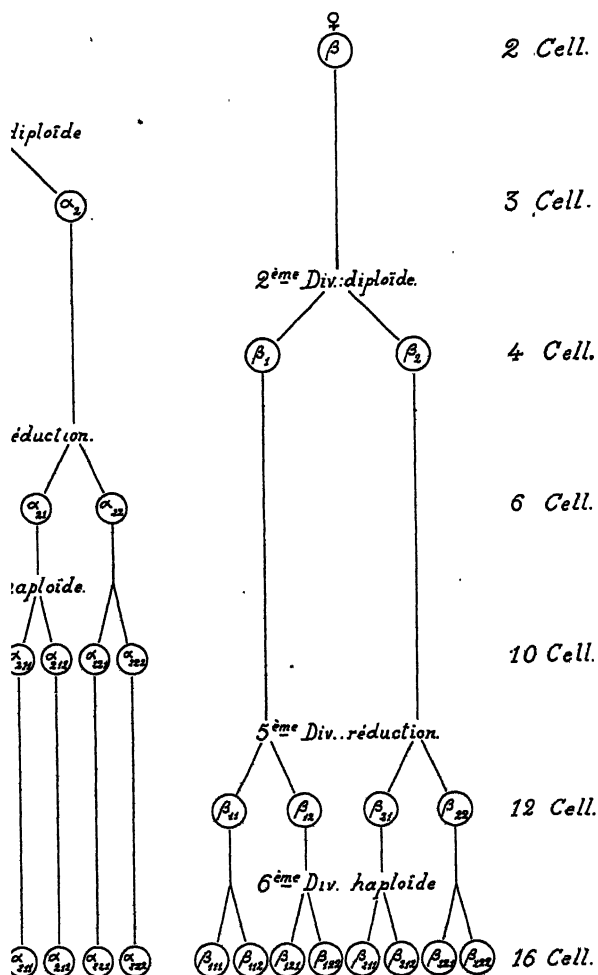
Le phénomène réductionnel ne fait donc pas de doute puisque l'état haploïde (deux chromosomes) est ainsi rétabli par une cinèse hétérotypique.

Sixième et dernière cinèse.

L'aspect de la seconde cinèse réductionnelle, ou division homéotypique de la lignée femelle, est comparable à celui que l'on observe au cours de la quatrième cinèse dans la lignée mâle. Les éléments cellulaires sont cependant légèrement plus gros. On n'observe à la prophase et durant la métaphase que deux chromosomes qui ne tardent pas à se diviser. Les figs. 19 et 20 (Pl. 32) montrent la migration vers les pôles qui reçoivent chacun deux éléments chromatiques.

A ce moment, les seize cellules gamétiques sont alors constituées. Après une courte phase de repos, les huit éléments mâles copuleront avec les huit éléments femelles, formant ainsi huit zygotes. Cependant, avant que la fécondation ait lieu, ces éléments s'accroissent jusqu'à ce que les cellules sexuelles aient atteint une certaine taille.

TEXT-FIG. 1.



représentant l'évolution des deux cellules germinales
t β) contenues dans le jeune pansporocyste.

NISME AU COURS DES DIVISIONS DU PANSPOROCYSTE.

ous l'avons vu à propos des figs. 14 et 15 (Pl. 32),
fois que la chronologie des diverses phases de la
porocyste soit légèrement altérée. Il peut se pro-

duire, par exemple, que le stade à quatre cellules au repos ne soit pas réalisé, la deuxième division étant immédiatement suivie de la troisième (cas de la fig. 14, Pl. 32). Un cas analogue est représenté à la fig. 15, où l'un des quatre cytes II de la lignée mâle ne s'est point encore divisé, alors que la cinèse réductionnelle de la lignée femelle est en pleine évolution.

Ces quelques cas, exceptionnels d'ailleurs, n'infirmen en rien les descriptions de Caullery et Mesnil, de Granata et de Mackinnon et Adam.

LA RÉDUCTION CHROMATIQUE DES ACTINOMYXIDIES.

Caullery et Mesnil, les premiers auteurs qui ont fait connaître avec précision le cycle d'une Actinomyxidie, ne parlent pas d'une réduction chromatique des gamètes. Ikeda, par contre, décrit l'expulsion de granules chromatiques dans les huit gamètes mâles, puis dans les huit gamètes femelles. Les figures qu'il donne (figs. 15, 16, et 17, Pl. 10) laissent subsister des doutes quant à la nature réductionnelle de ce phénomène. L'expulsion de gros granules par des noyaux quiescents semble exclure l'idée d'une réduction chromatique vraie. Dans mes coupes, j'ai souvent rencontré des pansporocystes de *Guyénotia* présentant nettement des signes de dégénérescence, et montrant, à côté d'une masse nucléaire sombre et granuleuse, un ou plusieurs gros granules chromatoides, sans doute expulsés du noyau dans le cytoplasme. Il est à remarquer que, dans ce cas, les pansporocystes se trouvaient presque toujours dans une position anormale, le plus souvent repoussés contre le chorion ou même dans le tissu chloragène. Il s'agit certainement de formes dégénératives dont je n'ai dès l'abord tenu aucun compte pour l'établissement du cycle de la *Guyénotia*. Ikeda admet donc que les granules chromatoides que l'on trouve à la fin de l'évolution du pansporocyste, entre les cellules germinales, ont la valeur de véritables globules polaires. Il en compte seize, chaque cellule gamétique en aurait donc expulsé un seul. Je crois que l'origine de ces granules chromatoides est toute autre; je pense en apporter la preuve dans le prochain paragraphe. Granata, par contre, décrit dans les gamètes des divisions

hétéropolaires qui aboutiraient à l'expulsion d'une masse chromatique hors du cytoplasme. Les figs. 37, 38, et 43 et son Mémoire en indiquent les principales phases. Je n'ai, pour ma part, jamais observé chez *Guyénotia sphaerulosa* d'aspects comparables.

GRANULES CHROMATOÏDES DU PANSPOROCYSTE.

On rencontre entre les cellules internes du pansporocyste un certain nombre de granules chromatoïdes sur l'origine desquelles subsiste un doute; Ikeda et Granata ainsi que Mackinnon et Adam les considèrent comme des globules de réduction. Cette interprétation me semble inacceptable :

1^o Dans les préparations fixées au liquide de Champy ou au mélange de Regaud IV, ces granules conservent d'une façon très élective l'hématoxyline, de même d'ailleurs que les nucléoles, à l'inverse de la chromatine au repos et en mouvement qui se décolore presque immédiatement au contact de l'alun de fer. Ceci nous indique une analogie de constitution entre les granulations chromatoïdes résiduelles et les nucléoles.

2^o Durant la cinèse, il est très fréquent de constater une migration du nucléole vers l'un des pôles cinétiques; migration qui précède l'ascension polaire des chromosomes et qui semble bien aboutir à une expulsion du nucléole hors de la cellule (voir figs. 16, 17, et 18, Pl. 32, et fig. 27, Pl. 33). Toutes les cinèses ne montrent pas une expulsion nucléolaire, mais de semblables phénomènes sont observables dans différentes phases, soit durant la gamétogenèse, soit durant la sporogenèse.

3^o L'existence de granules chromatoïdes résiduels s'observe déjà dans les pansporocystes à deux cellules internes (fig. 14, Pl. 32) et même avant (fig. 6, Pl. 32); on en observe à toutes les phases du cycle. Ce fait montre d'une façon indiscutable que ces globules n'ont rien de commun avec des globules de réduction.

4^o Enfin, de l'aveu même de Mackinnon et Adam, le nombre de ces granules n'est pas constant. Il est juste d'ajouter que ces derniers admettent qu'ils peuvent, dans certains cas, se

fusionner deux par deux, ce qui expliquerait — d'après ces auteurs — la fluctuation numérique.

Nous voyons donc, par ces quatre catégories de faits, que l'idée d'assimiler ces corpuscules à des corps de réduction est actuellement insoutenable. Le seul fait que les phénomènes réductionnels se font par des cinèses homopolaires semble d'ailleurs exclure cette interprétation.

LA FÉCONDATION.

Le pansporocyste, parvenu à maturité, présente donc seize éléments dont huit, légèrement plus petits, peuvent être assimilés à des gamètes mâles. Chacun de ces éléments est réduit et ne contient que deux chromosomes. Ces gamètes, après une période de repos, durant laquelle ils tendent à s'accroître, copulent deux par deux : un élément de taille plus réduite, s'accolant intimement à un gamète femelle. Il en résulte des 'copula' telles que celles figurées en 24 (Pl. 33). La plasmogamie se produisant, les noyaux sont mis au contact l'un de l'autre (fig. 25, Pl. 33), et ne tardent pas à se fusionner. Sitôt la copulation des deux noyaux achevée, le syncaryon du zygote ainsi formé laisse apparaître un spirème. La fécondation est donc légèrement anisogame, comme tous les auteurs l'avaient décrite.

LES DIVISIONS DU ZYGOTE.

Le noyau du zygote ou syncaryon entre très rapidement en cinèse après une courte période de repos. Dans la fig. 17 (Pl. 32), on aperçoit un noyau en prophase contenant un spirème déjà fragmenté en quatre éléments et un nucléole excentrique. La même figure montre un autre noyau métaphasique, avec quatre chromosomes en train de se fissurer. La fig. 26 (Pl. 33) représente également deux métaphases à quatre chromosomes, et les figs. 27 et 28 des anaphases. Durant cette première cinèse, les chromosomes sont de grande taille et bien observables. On remarque que deux grands et deux petits éléments émigrent à chaque pôle. Le noyau est donc typiquement diploïde. Cette première cinèse conduit à la formation de couples de deux

cellules, une plus grande et une plus petite, la cytodiérèse se montrant nettement hétéropolaire (voir fig. 26, Pl. 33).

La deuxième cinèse de la segmentation du zygote se produit tout d'abord dans le plus petit élément qui s'accolle au plus grand et entoure l'un de ses pôles à la manière d'un croissant. Cette deuxième cinèse montre quatre chromosomes métaphasiques (fig. 31, Pl. 33) et donne naissance à deux cellules en croissant qui enveloppent partiellement la grosse cellule centrale (fig. 31, Pl. 33 et fig. 29, Pl. 33). Ce n'est qu'à ce moment que cette dernière se divise à son tour, formant ainsi une troisième cellule externe, ceci par une cinèse hétéropolaire (fig. 29, Pl. 33).

L'ébauche de la spore est alors constituée par une grosse cellule centrale entourée de trois cellules plus petites qui l'enveloppent entièrement. Ces trois cellules ne sont autres que les trois cellules valvaires ou cellules de l'épispore, ou encore cellules sporales ou de l'involucre (voir fig. 23, 27, et 30, Pl. 33). Durant toutes ces cinèses, le noyau se montre toujours à l'état diploïde.

FORMATION DE L'ÉBAUCHE SPORALE, PUIS DE LA SPORE.

Caullery et Mesnil sont les premiers à avoir observé que la spore se forme à partir de deux masses indépendantes : l'une de six cellules, disposées près du centre du pansporocyste et l'autre, tout d'abord mononucléée, et périphérique. La première donnera les trois capsules polaires et les trois cellules valvaires initiales. Quant à la grosse cellule, elle est rejetée à la périphérie du petit kyste et fournira la masse des cellules germinatives. Mackinnon et Adam s'opposent aux conclusions de Caullery et Mesnil et n'admettent pas, d'après leurs observations sur *Triactinomyxon*, l'idée d'une ségrégation des deux futurs constituants de la spore (épispore et endospore). Par contre, chez *Sphaeractinomyxon gigas*, Granata confirme entièrement les observations de Caullery et Mesnil sur *S. stolci*, et il admet l'existence d'un pore, à la partie postérieure de l'involucre, par lequel la masse germinale pénètre à l'intérieur de la spore.

Quant à la généalogie des cellules qui formeront l'enveloppe

sporale (capsules polaires et cellules valvaires), il persiste un conflit entre les vues de Granata et celles de Mackinnon et Adam. Les schémas représentant la filiation des cellules sporales ne concordent pas d'après ces deux travaux. Remarquons d'ailleurs que les recherches de ces auteurs n'intéressent pas les mêmes genres.

Chez *Guyénotia sphaerulosa*, lorsque les trois cellules de la périphérie sont formées, la grande cellule centrale entre de suite en cinèse (fig. 23 et 30, Pl. 33). Chacun des deux noyaux résultant de cette division se divise à nouveau et l'on observe une cellule centrale à quatre gros noyaux entourée d'une pellicule formée de trois cellules qui se sont amincies et dont le noyau s'est aplati en diminuant de volume (fig. 34, Pl. 33). Ces noyaux périphériques entrent en cinèse (fig. 34, Pl. 33 en haut), pendant que les cellules enveloppantes se rétractent, ne couvrant plus qu'un pôle de la cellule centrale. Ils se trouvent alors au nombre de six noyaux de taille plus petite, tandis que les noyaux de la masse centrale atteignent le nombre de quatre. Cette sorte de calotte à six noyaux se projette souvent, dans les coupes, sur la masse centrale à quatre noyaux (fig. 32, Pl. 33). C'est à ce moment que se produit une sorte de décollement. La masse centrale tétranucléée est rejetée vers la périphérie du pansporocyste, alors que le plasmode à six noyaux se différencie en donnant trois cellules polaires dirigées vers le centre du kyste, en arrière desquelles s'observent les trois cellules valvaires initiales (ou cellules épisporales ou de l'involucre), dont les noyaux se gonflent peu à peu. C'est en arrière de cet ensemble où l'on distingue l'ébauche des trois capsules polaires et des trois valves, que se trouve la masse tétranucléée, dont les noyaux, subissant encore une division, atteignent le nombre de huit (voir fig. 39, Pl. 34).

Durant toute cette période, si les figures cinétiques ne sont pas trop petites, on observe toujours quatre chromosomes, soit le nombre diploïde. L'ébauche de l'involucre sporal subit alors une nouvelle division qui intéresse vraisemblablement les trois cellules valvaires. Il en résulte trois nouveaux noyaux qui restent directement en arrière des trois cellules polaires (fig. 40,

Pl. 34). Quant aux noyaux de la valve (fig. 40, Pl. 34), ils passent plus en arrière, et pénètrent dans trois appendices cytoplasmiques

TEXT-FIG. 2.

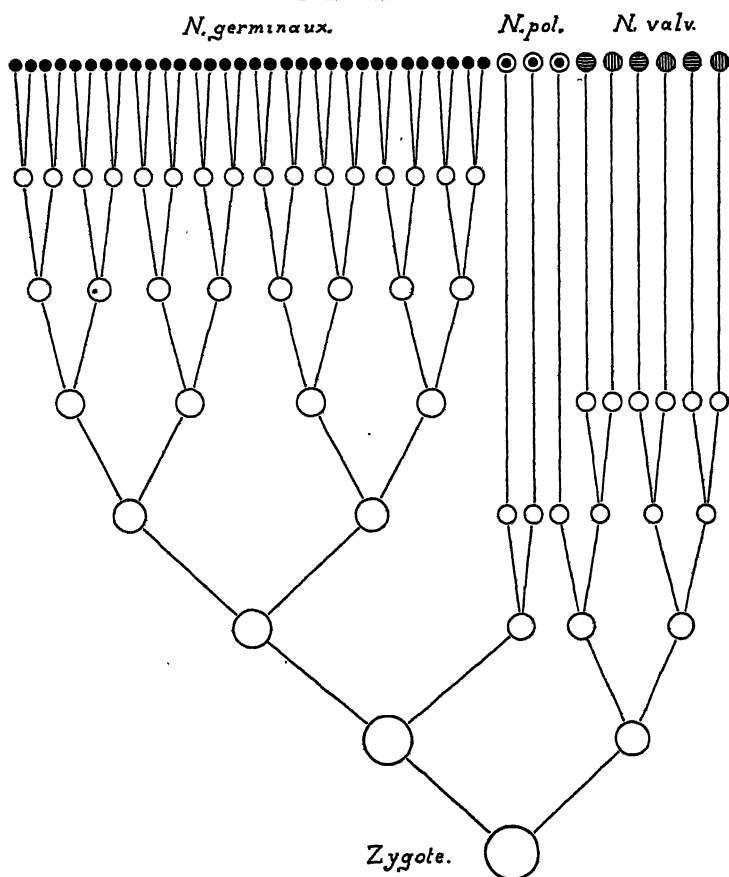


Schéma montrant la filiation des éléments de la spore à partir du zygote. *N. pol.*, noyaux polaires ; *N. valv.*, noyaux valvaires. De ces six noyaux, trois d'entre eux dégénéreront et émigreront au pôle antérieur de la spore.

ques, encore courts, qui s'accroissent du côté de la masse germinative. Un stade plus avancé montre l'accroissement des cellules valvaires qui constituent la paroi de la spore. Trois

travées cytoplasmiques, contenant les trois noyaux issus vraisemblablement de la division des noyaux valvaires, se prolongent en arrière du groupement polaire à la surface de la spore (fig. 38, Pl. 34). Ces noyaux émigreront, par la suite, au pôle antérieur de la cellule où ils semblent dégénérer (fig. 41, Pl. 34).

La masse germinative présente alors huit noyaux, elle pénètre à l'intérieur de cet involucre sporal, ou bien les cellules valvaires, en pleine période de croissance, l'enveloppent peu à peu. Il est difficile de décider lequel de ces deux processus — embolie ou épibolie — se produit effectivement. L'essentiel est de remarquer que la spore se referme alors complètement et contient une masse germinative à huit noyaux (fig. 35, Pl. 33). La fig. 36 montre l'aspect d'une spore vue de face avec ses trois capsules polaires, débouchant dans la commissure des trois valves. Les noyaux capsulaires sont encore observables. Par la suite, la spore s'agrandira, deviendra presque sphérique, les noyaux capsulaires disparaîtront, tandis que les noyaux de la masse germinative, par divisions successives, atteindront le nombre de trente-deux (Text-fig. 3).

Les cellules valvaires s'allongent alors, formant trois longs appendices de $40\ \mu$ de long, contenant le noyau valvaire au niveau du tiers postérieur. Ces trois grandes cornes s'accroissent jusqu'à la maturité de la spore (Text-fig. 3). Cette dernière mesure $14\ \mu$ à $15\ \mu$ de diamètre, et les capsules polaires atteignent $5\ \mu$ dans leur plus grand diamètre.

Un point, cependant, reste douteux. Comment se produit la plasmotomie de la masse germinative de la *Guyénotia*? Se forme-t-il trente-deux sporozoïtes mononucléés, ou bien, comme chez le *Tetractinomyxon* ou le *Triactinomyxon* légeri, la fragmentation cytoplasmique aboutit-elle à la formation d'éléments binucléés? Mes observations, tant sur frottis que sur coupes, ne m'ont jamais montré qu'une seule masse cytoplasmique indivise, contenant un maximum de trente-deux noyaux. Il est regrettable que cette lacune subsiste. Des observations précises sur ce point nous permettraient de comprendre l'origine des corps binucléés qui sont à la base de

TEXT-FIG. 3.

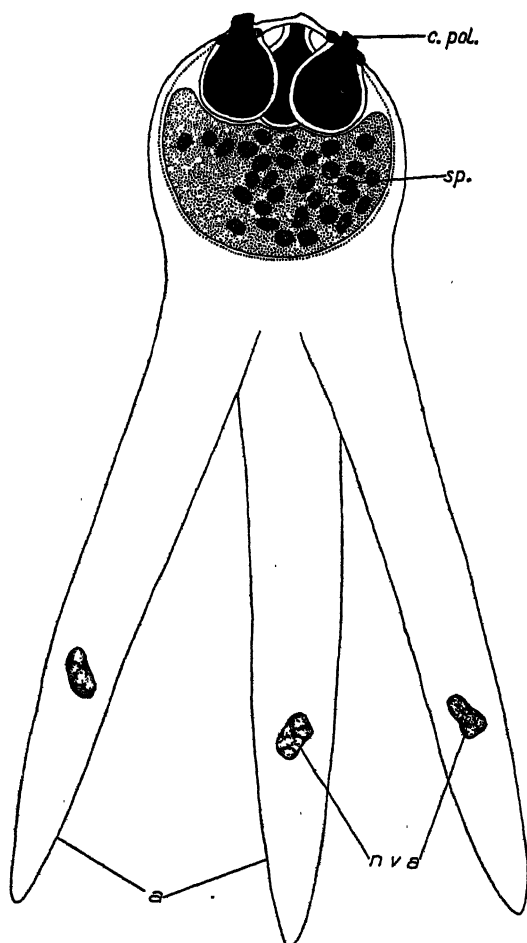


Figure demi-schématique représentant une spore mûre. (D'après un frottis coloré au Giemsa et d'après des coupes ; Grossissement $\times 3145$.) *a.*, appendices de la spore ; *c. pol.*, capsule polaire ; *nva.*, noyaux valvaires des appendices ; *sp.*, sporoplasme.

l'évolution du pansporocyste. Je n'ai pu, malheureusement, suivre cette formation des sporozoïtes et mes observations, à cet égard, sont incomplètes. Peut-être la spore n'achève-t-elle sa maturité que lorsqu'elle a été rejetée hors des tissus de l'hôte.

La figure schématique 2 représente la filiation des éléments de la spore comme j'ai cru pouvoir l'établir. Elle montre que chez *Guyénotia sphaerulosa* les processus essentiels de la formation de la spore sont comparables à ceux qui ont été décrits par Mackinnon et Adam chez *Triactinomyxon*, et par Granata chez *Sphaeractinomyxon gigas*. La généalogie des noyaux de la spore chez *Guyénotia* se distingue cependant de celle des genres précités par la formation de six noyaux valvaires, dont trois dégénèrent rapidement et par l'absence de noyaux résiduels de l'endospore. Ces deux faits montrent, à eux seuls, la différence essentielle existant entre le genre *Guyénotia* et les genres voisins.

CONCLUSIONS.

L'étude du cycle de *Guyénotia sphaerulosa* m'a permis de préciser un certain nombre de points du cycle évolutif des Actinomyxidies, en particulier les phénomènes réductionnels.

1° Les formes jeunes de *Guyénotia sphaerulosa* rencontrées dans les tissus de *Tubifex tubifex* Müll., qu'elles se trouvent dans l'épithélium intestinal, dans le tissu chloragène ou, accidentellement, à l'intérieur du coelome, sont toujours binucléées. Je n'ai donc pas pu décider si l'état binucléé provenait d'un sporozoïte lui-même binucléé (cas de *Tetractinomyxon Ikeda*), s'il résultait d'une division d'un noyau unique d'un sporozoïte mononucléé, ou si enfin l'accouplement de deux sporozoïtes mononucléés lui avait donné naissance.

2° L'évolution du pansporocyste, qui présente dans ce genre une paroi à quatre noyaux, suit exactement le mode décrit par Caullery et Mesnil, Mackinnon et Adam et, plus récemment, par Granata. Au cours de ce développement, la lignée germinale femelle manifeste une inertie cinétique très marquée par rapport à la lignée germinale mâle. Il résulte de cette évolution huit gamètes mâles et huit gamètes femelles, de taille inégale et qui, par leur accouplement, réalisent une fécondation faiblement anisogame.

3° Au cours de la formation des gamètes, on observe trois

cinèses successives dans chacune des deux lignées germinales. Dans chaque lignée (mâle ou femelle), la première cinèse est goniale (type diploïde à quatre chromosomes); la seconde cinèse est réductionnelle et hétérotypique (passage de l'état diploïde à l'état haploïde à deux chromosomes); quant à la troisième cinèse, elle est homéotypique et du type strictement haploïde (deux chromosomes). Une réduction chromatique précède donc la formation des gamètes; elle a lieu à l'avant-dernière cinèse. Les phénomènes prémeiotiques n'ont pas pu être suivis en détail.

4^o Les corpuscules chromatoïdes, rejetés à l'intérieur du pansporocyste, ne sont pas, comme le pensent Mackinnon et Adam ou Granata, des globules de réduction. Ils se rencontrent en nombre indéterminé et se forment par expulsion d'éléments d'origine nucléolaire à n'importe quelle phase de l'évolution du pansporocyste.

5^o La fécondation suivie de caryomixie, rétablit l'état diploïde du noyau (quatre chromosomes). Les cinèses, durant la sporogénèse, sont donc diploïdes.

6^o La filiation des noyaux de la spore semble se poursuivre à peu près suivant le schéma donné par Mackinnon et Adam pour *Triactinomyxon légeri*; cependant, chez *Guyénotia sphaerulosa*, il ne se forme pas de noyaux résiduels, et l'on rencontre six noyaux valvaires, dont trois sont appelés à dégénérer rapidement.

7^o La spore est formée, comme l'indiquent Caullery et Mesnil, par deux ébauches indépendantes: (1^o) Les cellules polaires et valvaires; (2^o) la masse germinative repoussée à la périphérie du pansporocyste. Cette dernière pénètre dans l'ébauche de l'épisporé, lorsqu'elle a atteint le nombre de huit noyaux. Je n'ai pu déterminer si cette pénétration de la masse germinative est active (embolique) ou si elle est due à l'enveloppement des cellules valvaires pendant leur croissance (épibolie).

8^o La constitution du sporozoïte (mononucléée ou binucléée) de *Guyénotia* reste à déterminer.

Les résultats acquis, concernant les cinèses maturatives et la fécondation de la *Guyénotia sphaerulosa* présentent un

intérêt certain. Comparés aux conclusions de mes dernières recherches sur les Myxosporidies ils montrent très nettement une homologie entre le cycle de ces deux groupes. La sexualisation plus précoce des Actinomyxidies conduit à une ségrégation immédiate des deux lignées germinatives mâle et femelle, entre lesquelles, d'ailleurs, les phénomènes réductionnels présentent une rigoureuse similitude. Comme je l'ai montré dans mon récent travail sur les Myxosporidies, la sexualisation de plus en plus précoce que l'on observe dans les divers groupes de Myxosporidies doit conduire finalement à la dioecie. Si, chez les Actinomyxidies, la dioecie n'est point encore réalisée, la très grande précocité de la sexualisation conduit à la production de cinèses goniales dans des éléments déjà déterminés dans le sens mâle ou dans le sens femelle. C'est donc bien à l'avancement de la sexualisation par rapport au processus réductionnel que semble due l'allure particulière du cycle des Actinomyxidies.

INDEX BIBLIOGRAPHIQUE.

1. Auerbach, M.—'Die Cnidosporidien.' Leipzig, 1910.
2. Caullery, M. et Mesnil, F.—"Sur un type nouveau (*Sphaeractinomyxon stolci* n.g., n.sp.) d'Actinomyxidie et son développement" 'C. R. Soc. Biol., Paris', vol. 56, 1904.
3. ——— "Sur les affinités des Actinomyxidies", *ibid.*, 1904.
4. ——— "Recherches sur les Actinomyxidies. I. *Sphaeractinomyxon stolci*, C. et M.", 'Arch. f. Protistenk.', Bd. 6, 1905.
5. Doflein, F.—'Lehrbuch der Protozoenkunde', 4^e Aufl. Jena, 1916.
6. Granata, L.—"Ricerche sugli Attinomissidi. I. *Triactinomyxon magnum* n.sp.", 'Mon. zool. ital.', anno 33, n° 11, 1922.
7. ——— "II. *Neactinomyxon globosum* n.g., n.sp.", *ibid.*, anno 33, n° 12, 1922.
8. ——— "III. *Sphaeractinomyxon gigas* n.sp.", *ibid.*, anno 34, n° 4, 1923.
9. ——— "IV. Osservazioni sullo sviluppo di *Sphaeractinomyxon gigas* Gr.", *ibid.*, anno 34, n° 9, 1923.
10. ——— "Gli Attinomissidi; Morphologia, Sviluppo, Sistematica", 'Arch. f. Protistenk.', Bd. 50, 1924.
11. Hartmann, M.—"Sporozoa"; in Kükenthal's 'Handbuch der Zoologie'. Berlin & Leipzig, 1923-5.
12. Ikeda, I.—"Studies on some Sporozoan parasites of Sipunculoïds. I. The life-history of a new Actinomyxidian, *Tetractinomyxon intermedium* g. & sp. nov.", 'Arch. f. Protistenk.', Bd. 25, 1912.

13. Léger, L.—'C. R. Congr. Assoc. fr. Av. Sc., Anger', 1903.
14. ——"Sur la sporulation du *Triactinomyxon*", 'C. R. Soc. Biol., Paris', vol. 56, 1904.
15. ——"Considérations sur le genre *Triactinomyxon* et les *Actinomyxidies*", *ibid.*, vol. 56, 1904.
16. Mackinnon, D. L. et Adam, D. T.—"Notes on Sporozoa parasitic in *Tubifex*. I. The life-history of *Triactinomyxon Stolc*", 'Quart. Journ. Micr. Sci.', vol. 68, Part II, 1924.
17. Mrázek, A.—"Analyse du Travail de Stolc", 'Zool. Centralbl.', Bd. 7, 1899.
18. Naville, A.—"Recherches sur la sexualité chez les *Myxosporidies*". 'Arch. f. Protistenk.', Bd. 69, 1929.
19. Stolc, A.—"Actinomyxidies, nouveau groupe de Mésozoaires, parent des *Myxosporidies*", 'Bull. inter. de l'Acad. d. Sc. de Bohême', 1898.
20. Wenyon, C. M.—'Protozoology.' London, 1926.

EXPLICATION DES PLANCHES.

N.B.—Toutes les figures ont été dessinées à la même échelle, en utilisant des esquisses faites au moyen de la chambre claire d'Abbe (ocul. peripl. Leitz $\times 25$ et immers. homog. Leitz 1/12ème). Ces figures ont été réduites, à la reproduction, exactement d'un tiers. Le grossissement est donc de 3.145 diamètres. Les préparations ont été colorées à l'hématoxyline de Heidenhain et fixées au liquide de Bouin-Hollande ou à celui de Duboscq et Brasil.

PLANCHE 32.

Fig. 1 à 4.—Petits corps binucléés dans les cellules épithéliales ou chloragogènes du *Tubifex*.

Fig. 5.—État spirémique des deux noyaux des corpuscules binucléés.

Fig. 6.—Corps à trois cellules.

Fig. 7.—Élément à quatre cellules.

Fig. 8 et 9.—Enveloppe du pansporocyste présentant déjà quatre cellules et contenant les deux cellules mères de la lignée germinale.

Fig. 10.—Pansporocyste renfermant quatre éléments équivalents aux cytes de premier ordre.

Fig. 11.—Cellule goniale α en cinèse à côté de la cellule β en prophase.

Fig. 12.—Pansporocyste contenant les éléments α_1 , α_2 , β_1 , β_2 .

Fig. 13.—Télophase donnant naissance aux éléments α_1 et α_2 , et métaphase cinétique de la cellule β .

Fig. 14.—Anaphase de la cellule β et télophase ou prophase des éléments α_1 et α_2 .

Fig. 15.—Prophase et anaphase des éléments β_1 et β_2 , la cellule α_{22} (ou une homologue) n'est point encore divisée.

Fig. 16.—Métaphase de la deuxième cinèse réductionnelle de la lignée femelle.

Fig. 17.—Prophase et métaphase de la première division du zygote.

Fig. 18.—Anaphase de la première cinèse réductionnelle de la lignée mâle.

Fig. 19.—Anaphase de la deuxième cinèse réductionnelle de la lignée femelle.

Fig. 20.—Deux anaphases de la deuxième cinèse réductionnelle de la lignée femelle.

Fig. 21.—Pansporocyste à dix cellules, contenant les éléments : α_{111} , α_{112} , α_{121} , α_{122} , α_{211} , α_{212} , α_{221} , α_{222} , β_1 et β_2 .

PLANCHE 33.

Fig. 22.—Prophase de la deuxième cinèse réductionnelle de la lignée mâle.

Fig. 23.—Prophase et métaphase de la première cinèse de la lignée germinale de l'ébauche sporale. Élément germinatif central accompagné des trois cellules de l'enveloppe.

Fig. 24.—Diverses phases de la copulation des gamètes.

Fig. 25.—Caryogamie des pronuclei mâles et femelles.

Fig. 26.—Première cinèse de division du zygote : deux métaphases et deux éléments déjà divisés.

Fig. 28.—Anaphase de la première division du zygote.

Fig. 29.—Deuxième cinèse de la cellule centrale de l'ébauche sporale.

Fig. 30.—Prophase et métaphase de la première cinèse de la lignée germinale de l'ébauche sporale.

Fig. 31.—Division de la première cellule de l'involucre de l'ébauche sporale. Stade à trois cellules.

Fig. 32.—Ébauche sporale montrant les six noyaux de l'involucre et la formation des quatre noyaux germinatifs.

Fig. 33.—Divers aspects de l'ébauche sporale.

Fig. 34.—Ébauche sporale montrant des métaphases à quatre chromosomes.

Fig. 35.—Jeune spore contenant un sporoplasme à huit noyaux (sur coupes).

Fig. 36.—Jeune spore en vue polaire montrant les commissures valvaires et les trois capsules polaires accompagnées de leurs noyaux (sur coupes).

Fig. 37.—Spore vue en coupe. (Il est à remarquer que dans les coupes les appendices valvaires restent incolores et ne peuvent être observés.)

PLANCHE 34.

Fig. 38.—Évolution de l'involucre d'une spore montrant les six noyaux valvaires (d'après un frottis coloré au Giemsa).

Fig. 39.—Phase initiale de la formation de l'involucre sporal, montrant les trois capsules polaires avec leurs noyaux et les trois noyaux initiaux des valves de la spore (sur coupes).

Fig. 40.—Stade jeune de la formation de l'involucre sporal, montrant l'existence de six noyaux valvaires (d'après un frottis coloré au Giemsa).

Fig. 41.—Jeune spore contenant un sporoplasme à seize noyaux. Les noyaux valvaires antérieurs ont émigré au pôle antérieur de la spore où ils ne tarderont pas à dégénérer (d'après un frottis coloré au Giemsa).



1



3



5



6



2



4



7



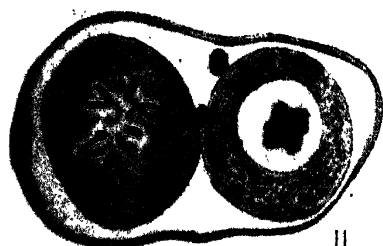
8



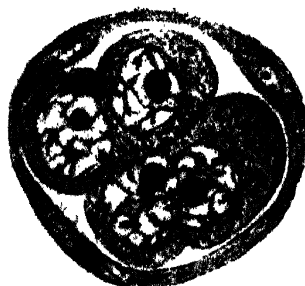
9



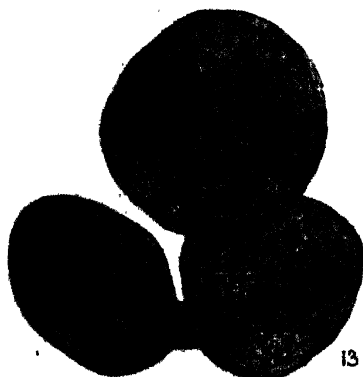
10



11



12



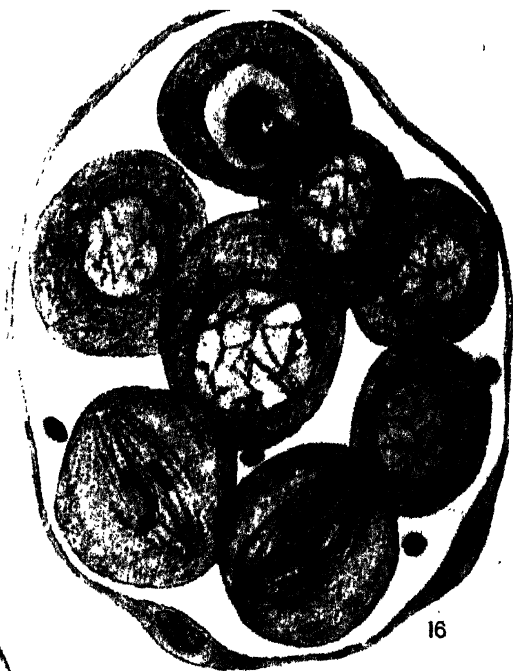
13



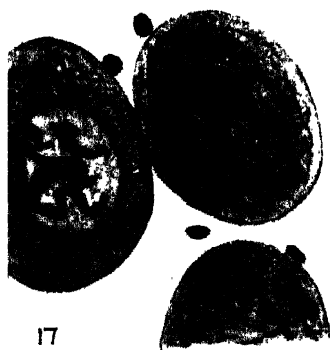
14



15



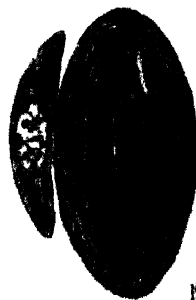
16



17



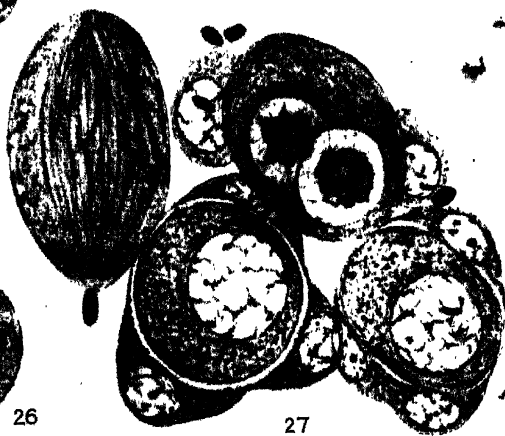
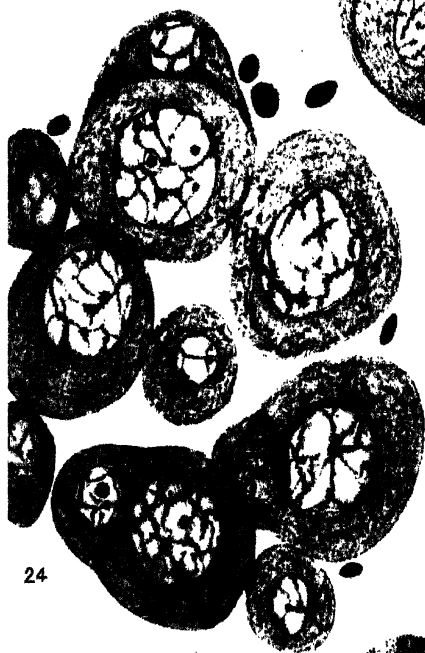
18



19

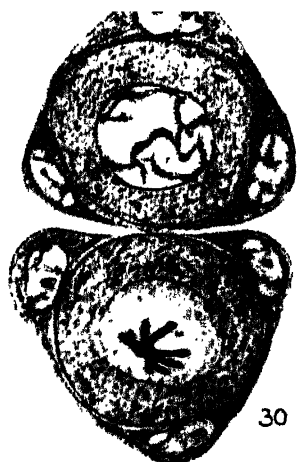


21

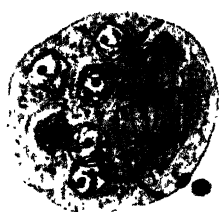




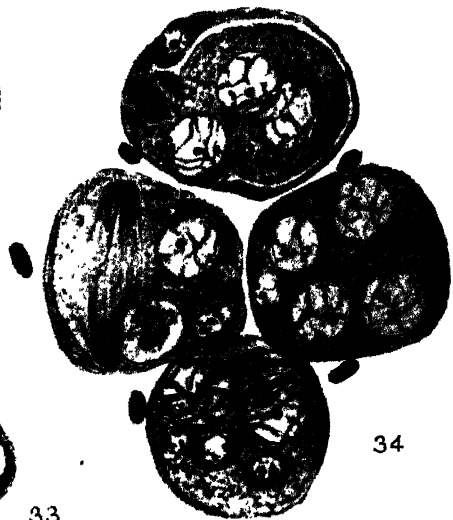
29



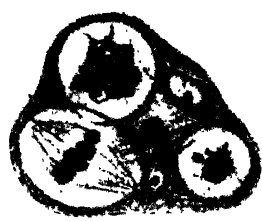
30



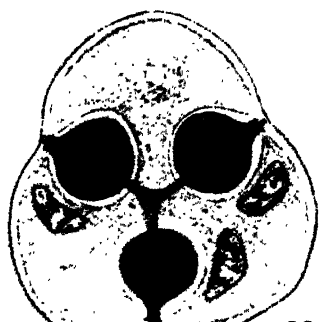
32



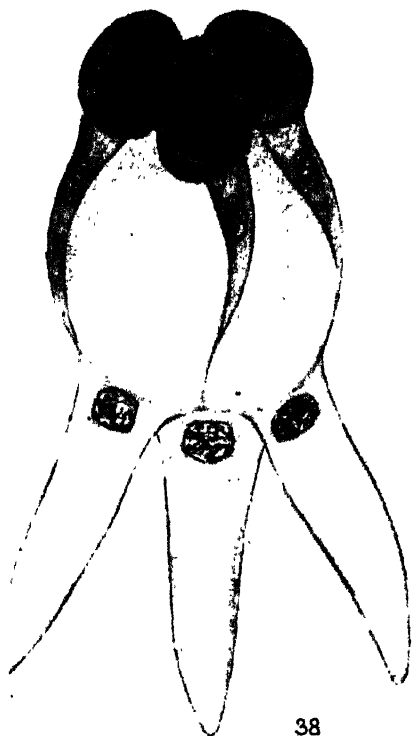
34



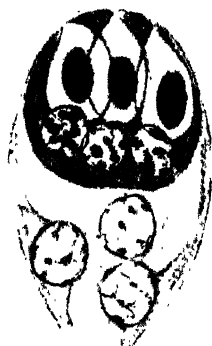
33



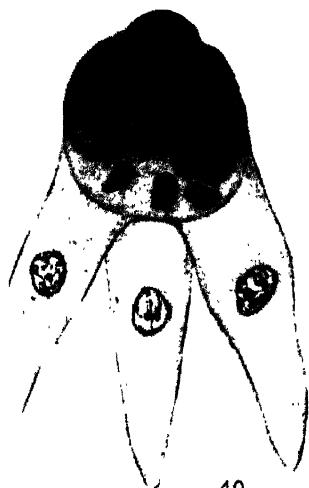




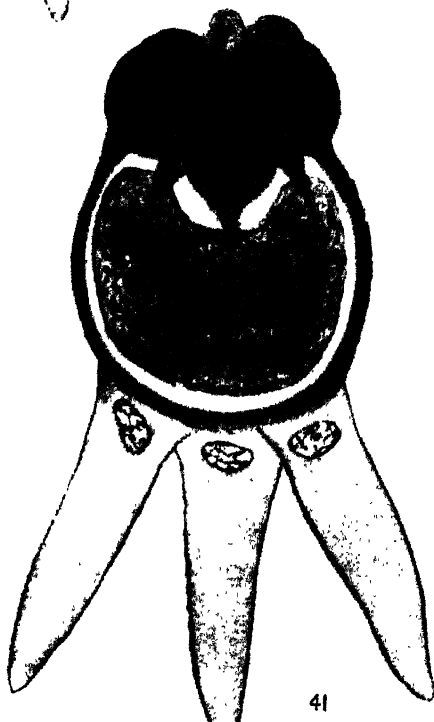
38



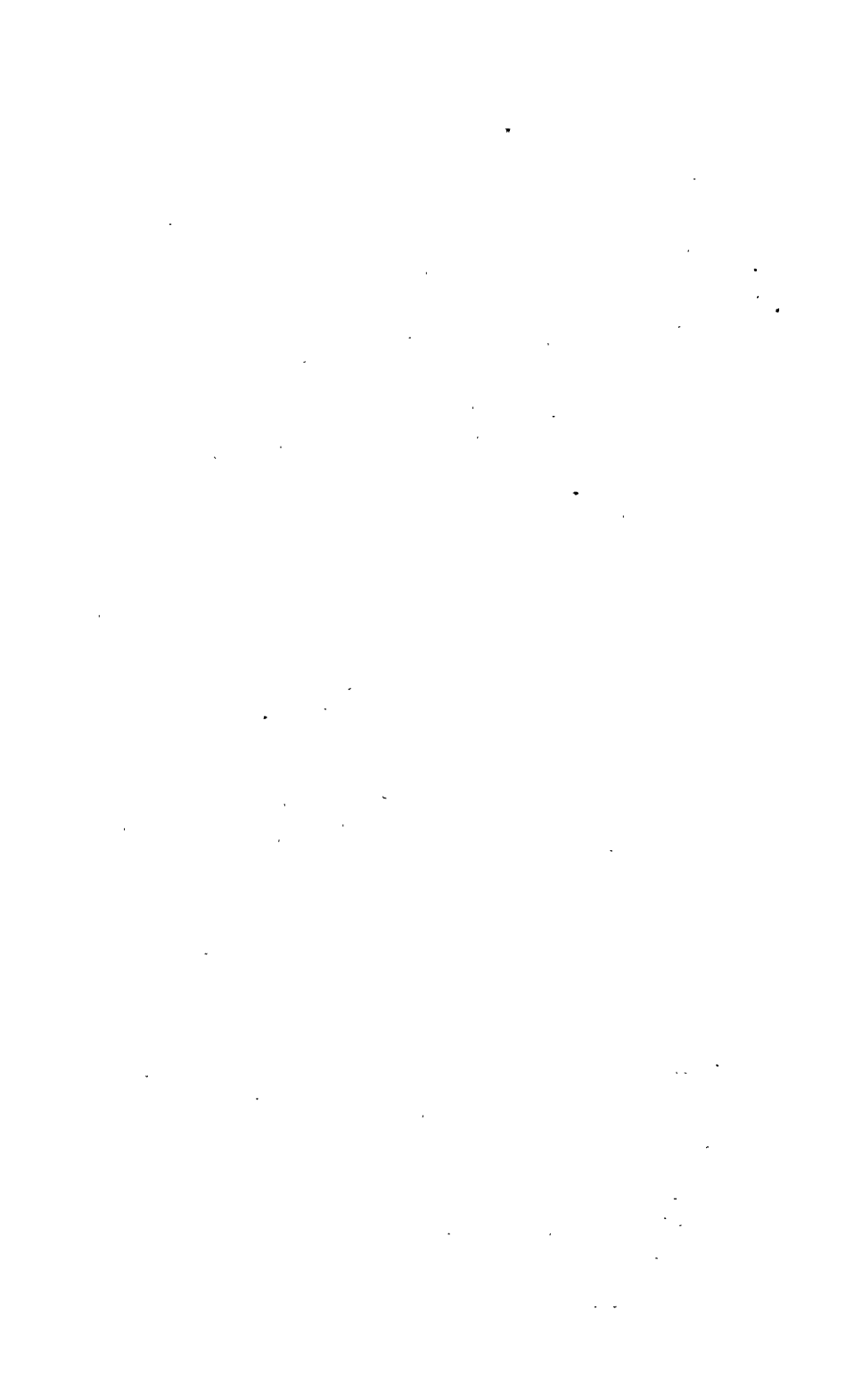
39



40



41



Seasonal and Sexual Variation in the Thyroid Glands of Cats.

By

Esther Lowe, M.Sc.

Assistant Lecturer in Zoology, University of Manchester.

With Plates 35-36.

CONTENTS.

	PAGE
1. INTRODUCTION	577
2. MATERIAL AND METHODS	578
3. INFLUENCE OF SEASON ON HISTOLOGICAL STRUCTURE	578
4. INFLUENCE OF SEX ON HISTOLOGICAL STRUCTURE	585
5. INFLUENCE OF CASTRATION	586
6. INFLUENCE OF PREGNANCY AND LACTATION	587
7. SUMMARY	590
8. REFERENCES	591
9. EXPLANATION OF PLATES	591

INTRODUCTION.

AN account of the variation in the histological condition of the thyroid gland of the sheep, with regard to age, sex, and season, has already been published (Lowe, 1930). The results embodied in the present paper formed part of the same investigation.

The main part of the investigation, dealing with sheep, was undertaken originally on the advice of Mrs. Bisbee in connexion with a suggestion she put forward (Bamber, 1, 1924) as to the possible relation between Braxy in sheep and seasonal activity of the thyroid.

It was suggested by Professor Dakin, however, that it would be of interest to include in the investigation the thyroid glands of cats also, as they are animals whose sexual period falls within more definite limits.

The laboratory work was carried out in the Zoological Department of the University of Liverpool during the year October 1924-5. I am indebted to Professor Dakin and to Mrs. Bisbee for suggesting the work originally and for much valuable advice and encouragement during the course of the investigations.

The cats' glands used in this investigation were obtained daily from freshly killed animals and were placed in the fixative within about half an hour of the death of the animal, so that post-mortem changes were as far as possible eliminated. It was, of course, impossible to ascertain the ages of the cats used, but the sex was recorded in each case. Glands from males and females have been taken in approximately equal numbers throughout. A few castrated animals were used also, and during the late winter and spring months, numerous females in different stages of pregnancy were obtained, as well as a few in the lactation period.

MATERIAL AND METHODS.

The glands, being small, have been fixed whole throughout the investigation, as this practice facilitated the orientation of the paraffin blocks later. The sections were cut longitudinally in all cases. The material was fixed in Bouin's picro-formol for the most part. This fixative was found quite successful for the glands of cats, although useless for those of sheep.

The chief stains used have been Mann's methylblue eosin and Mallory's triple stain.

THE THYROID GLAND OF THE CAT.

INFLUENCE OF SEASON ON HISTOLOGICAL STRUCTURE.

In the case of cats a fairly complete series of glands from both males and females has been obtained throughout the whole year, so that it has been possible to obtain a much more complete account of the seasonal variation in the thyroid glands of cats than was possible in the case of sheep. The fact, however, that it was never possible to ascertain the ages of the cats whose glands were used makes this account in some respects less exact than that given for sheep.

For convenience the results in this section will be discussed under the periods October–December, January and February, March–May, and June–August.

October–December (inclusive).

On the few occasions on which large numbers of animals were taken in one week there was remarkably little individual variation in their histology. For instance, in the week October 24–30, the glands of eleven cats were obtained. One of these was from a castrated male and will not be discussed here. Five were from females, and all were remarkably similar in condition. Five others were from males, and these, although differing considerably from those of the females, were very similar amongst themselves.

The glands from the five females all contained very small acini with extremely small amounts of colloid (fig. 1, Pl. 35). Most of the glands were producing a certain amount of new secretion and the acinar epithelium was in most cases low columnar. The acini themselves were very small and were composed of few cells (fig. 1, Pl. 35). Many of them were completely devoid of colloid, but the epithelium showed few signs of having undergone any process of compensatory hypertrophy. The acinar walls were in most cases one cell thick, and in those areas of the gland from which colloid was absent the cell-masses seemed to be the remains of empty acini rather than the result of a process of active cell-division.

The glands of the five males, on the other hand, contained considerably more colloid (fig. 2, Pl. 35), and the acini were much larger. In one or two cases the epithelial cells were flattened, but, notwithstanding this, a certain amount of new secretion was being formed. One of the glands showed a more actively secreting condition than the other four. The acini were smaller and the epithelial cells cuboidal or columnar in shape with a tendency to active cell-division. A considerable amount of new secretion was present. The other four glands obtained on the same date were fairly uniform in condition (fig. 2, Pl. 35).

The glands of most of the male cats obtained during the

months of October, November, and December were similar in condition to the four described above. They contained fairly large amounts of colloid, but unlike the glands of the male sheep examined during the same period, they all contained a certain amount of new secretion, and none of them could be described as colloid goitres. There was never any thickening of the connective tissue between the acini, but one or two of the glands showed signs of previous hypertrophy. This was particularly evident in one of the glands obtained on November 24. In this gland the acini were very large and showed plicated walls. The epithelium was low cuboidal or flat in character.

The glands of the male cats were not as uniform in condition as those of the sheep during the same period, and several exceptions were seen in which the amount of colloid was reduced. This was accompanied in some cases by active secretion, but in others the acini were quite small but contained no trace of new secretion, and seemed to be in an inert condition.

During the same three months the glands of numerous female cats were examined, and it was found that all the glands contained very small acini as in the case of the females obtained on October 24-30, described above, although the amount of new secretion was not the same in all cases. During October and the early part of November the amount of new secretion produced was rather small in comparison with the reduced size of the acini, but the glands obtained during late November and December were in a more actively secreting condition. The epithelium became more columnar in character, and in some cases a certain amount of hypertrophy was observed. There was not, however, any increase in the size of the acini during this period.

Thus during the autumn months the glands of both sexes were producing a certain amount of new secretion, but the amount of colloid was much greater in males than in females.

January and February.

During the following two months, January and February, the condition of the ordinary males and females became more

similar (figs. 3, 4, and 5, Pl. 35). In the females the acini became considerably larger and were producing much more new secretion than in the preceding three months (fig. 5, Pl. 35). The epithelial cells were in most cases columnar, and in most of the glands the acinar walls showed a tendency to hypertrophy. This actively secreting condition was accompanied by a marked distension of the capillaries of the gland. A number of females obtained during January and February were pregnant, but the condition of their thyroids fell into line more or less with that of the non-pregnant females. They will, however, be discussed in detail in a separate section. Towards the end of February and the beginning of March, the amount of colloid in the acini was somewhat reduced again, but the glands were in most cases still actively secreting, and some showed evidences of recent hypertrophy in the form of irregular acini, active cell-division, and thickened connective tissue.

The glands of the males during January and February also became more actively secreting than they had been during the autumn months. In most cases this condition of active secretion resulted in hypertrophy and cell-multiplication, particularly towards the end of February. The acini in many cases were smaller than those of the glands taken during the autumn months. In one gland obtained on February 18 the acini were completely exhausted and devoid of colloid, and the acinar walls had undergone a considerable amount of cell-division, but the gland of another male obtained during the same week had not arrived at such an extreme condition, and although it showed abundant traces of hypertrophy, a fair amount of colloid still remained (fig. 4, Pl. 35).

During January and February, therefore, the glands of both sexes were very similar in condition, both being actively secreting with a fair amount of colloid in the acini. The amount of colloid in the females was considerably larger than during the autumn months.

March, April, and May.

During March, April, and May, the glands of both sexes

became much more passive in condition (figs. 7, 8, and 10 Pl. 36). The amounts of colloid in the acini became much larger, although it was mostly of a very new faintly staining granular nature. The acinar epithelium was in most cases low cuboidal, that of the males being flatter than that of the females. The masses of cells formed during the previous period of activity were very conspicuous. There was also a certain amount of cell-debris in many of the acini. The epithelial cells in practically all the glands of both sexes during this period were in a resting condition and were not producing globules of new secretion.

The condition described above lasted until the end of May in both sexes. Only males and pregnant females were obtained during the first week in June, but the males were still in the same condition.

The only exceptions to the general rule during this period (March to first week in June) were the glands obtained from a few pregnant females. These glands resembled those of the non-pregnant females in the possession of large amounts of colloid in the acini, but they differed in that they were still producing a considerable amount of new secretion. In addition to the enlargement of the pre-existing acini found in the glands of all the females during this period, in the pregnant females a great many small new acini were being formed by the production of colloid in the middle of the small groups of cells which arose during the previous period of hypertrophy.

June—August.

From about June 10 onwards the condition of the glands again became more active. The glands of both sexes obtained on this date showed a great increase in activity.

This return from the resting to the active condition was foreshadowed by a tendency to slight activity in the glands of a few of the males towards the end of May. One gland obtained on May 26, for instance, showed small amounts of new secretion in various regions. The gland contained a moderate amount of colloid, and the acinar epithelium varied from a flat to a cuboidal

condition. There was abundant evidence of previous hypertrophy. The complete return to the active condition in all the glands, however, did not take place until the second week in June. The few glands mentioned above were merely of sporadic occurrence, and in the first week in June no active glands were obtained.

The two glands obtained from males between June 10 and 13 were in a very actively secreting condition. The acini were reduced in size; one was almost devoid of stainable colloid, and in the other the colloid was basophil and stained very lightly with methyl blue. In both glands the epithelium was either high cuboidal or columnar in condition and was in a state of active hypertrophy. In the more active of the two it was high columnar throughout. The other gland contained one or two very large acini which did not take part in the general activity. In both glands the capillaries between the acini were distended with blood.

The gland of the female obtained on June 13 was in a state of active hypertrophy with a considerable amount of cell-division in the acinar walls. The size of the acini was extremely varied throughout the gland. Some were very large and contained deeply staining colloid and a flat epithelium and very little new secretion; others were considerably reduced in size and were lined by actively secreting columnar epithelium. The connective tissue was thickened in places, and throughout the gland the capillaries were dilated. Its condition on the whole resembled that of the two males described above.

Most of the glands obtained between June 10 and the middle of August, when the last glands of the series were taken, conformed more or less to the description given above for the two sexes (figs. 11 and 12, Pl. 36). With the exception of pregnant females and castrated males, which are not being discussed here, the glands of both sexes were on the whole characterized by the production of large quantities of new secretion. The secreting cells were in nearly all cases high columnar and vacuolated and the nuclei were situated at the base of the cells. Practically all the glands contained enlarged capillaries. Hypertrophy and

cell-proliferation accompanied this condition of activity in some of the glands, but this was not universal.

One or two exceptions to the general description given above occurred among the males. These agreed with the account given above in that they contained much less colloid than the glands of either sex during the preceding period, March-May, but they differed from the glands of other males obtained during the period June-August in that they were producing very little new secretion, and the epithelial cells were either low cuboidal or flat in shape.

It is known from the experiments of Mills with the glands of guinea-pigs that an increase of temperature induces the colloid condition, whereas a decrease of temperature causes increased activity. The existence of the colloid condition in the glands of sheep of both sexes during the summer months (Lowe, 1930) is in accordance with Mills's (6, 1918) results for guinea-pigs. In the case of cats, however, the glands of both sexes were found to be actively secreting during the summer months. Their behaviour, therefore, was not in accordance with Mills's account.

Thus from the results discussed in detail above it is clear that there is a definite seasonal variation in the activity of the thyroid gland of cats. During the autumn months, the amounts of colloid were much larger in males than in females, but both sexes were producing small amounts of new secretion.

From the beginning of January onwards the amount of colloid in the two sexes was very similar and the seasonal changes which took place affected males and females equally. During January and February the glands of both sexes contained a moderate amount of colloid. A great increase in the activity of the glands took place early in January and lasted until March, when it was replaced by a more passive condition accompanied by an increase in the amount of colloid. This was again replaced at the beginning of June by another period of activity which lasted until the middle of August.

Thus the glands of ordinary males and females appear to respond simultaneously to seasonal changes during the greater

part of the year. The sex difference found in the glands during the autumn was comparable to that found in sheep (Lowe, 1929), but was not as pronounced. The period of activity in the spring months was found to set in earlier in cats than in sheep, but whereas in sheep it lasted until the beginning of May, in cats it was replaced in March by a period of inactivity which lasted until early June, when the gland's activity was renewed.

The beginning of activity in the glands of cats early in January synchronizes with the onset of sexual activity and may be connected with it. The return of the thyroid to the resting condition in March and the renewal of its activity in June are not, however, connected with any clearly defined sexual phase.

INFLUENCE OF SEX ON HISTOLOGICAL STRUCTURE.

During the greater part of the year it was found that the thyroids of the ordinary males and females differed very little in their histological condition. The changes which took place at various seasons and with different conditions of sexual activity seemed to affect the two sexes simultaneously. There was, however, throughout the year, more individual variation in the glands of the males than in those of the females. The only part of the year in which there was a well-marked sexual differentiation in the glands was the autumn period including October, November, and December. Most of the glands from male cats obtained during this period contained large amounts of colloid in their acini, whereas in the females the acini were considerably reduced in size, and in most of the glands there were areas from which colloid was absent. One or two of the female glands were entirely devoid of stainable colloid. The acini in the females were composed of few cells, and the reduction in the amount of colloid was not accompanied by compensatory hypertrophy of the epithelium. The epithelium in the females was low columnar in character; in the males it was either flat or low cuboidal. The amount of new secretion produced was, however, somewhat similar in the two cases. The amounts of new secretion became increased towards the end of November and the beginning of December. The capillaries were not dilated in any of the glands

during this period, nor was there any thickening of the connective tissue.

From the end of December onwards there was practically no difference in the histological condition of the glands of the two sexes. The condition of both has been described in the section dealing with seasonal variation, and as there is no sexual variation it is not proposed to discuss them again here. The remainder of the present section will deal with the influence of castration on the thyroid of the male, and of pregnancy and lactation on that of the female.

INFLUENCE OF CASTRATION.

Only four glands were obtained from castrated animals, but these were all obtained at different seasons of the year. Their condition did not on the whole agree with that of the males obtained on or about the same dates.

The first was obtained on October 29. Its condition resembled that of the females obtained in the same week much more closely than the condition of the males (see p. 579). The gland contained very little colloid, and the acini were reduced in size, some of them being entirely devoid of colloid. The epithelium was cuboidal in condition and was producing a fair amount of new secretion. There was a certain amount of cell-division in the acinar walls, and the capillaries of the gland were distended. The gland, therefore, was in a more actively secreting condition than those of either the males or the females obtained during the same week.

The second gland from a castrated animal was obtained on November 10. The condition of this gland was quite different from that of the preceding one, and from those of the females obtained on the same date. Its condition resembled that of the normal male. The acini contained large amounts of colloid. There was no sign of hypertrophy, the acini being lined by a single layer of low cuboidal cells which were producing small amounts of new secretion.

The third gland was obtained on January 13 and resembled the first in the possession of very little colloid. The acini varied

somewhat in size, but some of them were completely devoid of colloid. The acinar epithelium was low columnar in character and appeared to be in a condition of hypertrophy. Globules of new secretion were being formed, but the gland as a whole contained very little stainable colloid. The glands of the normal males and females obtained about the same time contained much more colloid and were producing more new secretion.

The remaining gland was not obtained until June 30 and was in a goitrous condition. The acini contained very small amounts of acidophil colloid. The epithelial cells were mostly columnar in shape and were secreting actively. Active cell-division was in progress, and some of the acini were exhausted and remained merely as cell-masses. The connective tissue throughout the gland was greatly thickened and contained elliptical nuclei. The condition of this gland was in harmony with the findings for normal males and females obtained during the same period, except that, in addition to being in a state of active secretion, it was goitrous and its colloid was eosinophil instead of basophil.

Thus the four glands described above did not seem to fall into line with the seasonal variation observed in normal males and females, neither did they resemble one another. If a larger number had been examined possibly further light would have been thrown on the subject, but certainly the present data provide no clue to any definite variation.

INFLUENCE OF PREGNANCY AND LACTATION.

During the year the glands from ten pregnant females have been examined. Only one of these was obtained during the autumn months; the others were obtained periodically throughout the rest of the year.

The gland obtained on November 7 agreed with those of the normal females obtained on the same date in the possession of small acini containing very little colloid, but the gland of the pregnant female was in a state of active hypertrophy, whereas those of the normal females were much less active and did not show hypertrophy.

The second gland obtained on January 27 resembled those

of the normal females obtained on the same date. It contained large acini filled with somewhat granular colloid. The gland was actively secreting and there was a certain amount of cell-division in the acinar walls. The capillaries of the gland were greatly distended.

The gland obtained in the following week resembled those of normal females obtained on the same date in that it was very actively secreting. The acini were irregular in shape and the epithelial cells were columnar and highly vacuolated. The whole gland was in a condition of hypertrophy with thickened connective tissue and enlarged capillaries. Fibroblasts were present in increased numbers in the connective tissue.

Two more glands were obtained on February 23. Both were actively secreting like those of the normal females obtained at the same time, but the glands of the pregnant females were producing more new secretion than those of the normal females. One of the glands contained more colloid than the other, and this, although very actively secreting, showed no signs of hypertrophy. In both glands the epithelial cells were columnar and the capillaries were dilated (fig. 6, Pl. 35).

Another gland was obtained from a pregnant female on March 2. This resembled the more active of the two glands obtained from pregnant females on February 23, and was producing more new secretion than the glands of normal females obtained about the same date. The epithelial cells were columnar and the capillaries were dilated.

Thus during January and February the glands obtained from pregnant females were all actively secreting, although the amount of colloid was somewhat varied. The degree of compensatory hypertrophy was also variable. The glands of the normal females during the same period were also in an actively secreting condition, but on the whole the glands of pregnant females contained less colloid and more new secretion than those of non-pregnant females.

The glands of three pregnant females obtained on March 14, May 27, and June 3 respectively, all contained large acini with large amounts of colloid and very little new secretion, and in

this respect bore a stronger resemblance to the glands of normal females obtained during the same period than to those of pregnant females obtained during January and February. The normal females, however, were practically devoid of new secretion. The epithelium in the three pregnant females was cuboidal, and there was no hypertrophy but the capillaries were enlarged. One female was in a much earlier stage of pregnancy than the other two, yet the glands of all were similar, so that the difference between these and the ones obtained in January and February was evidently not due to a difference in the stage of pregnancy.

The last pregnant female of the series was obtained on July 1. This gland contained larger acini and more colloid than the glands of normal females obtained during June and July, but it resembled them in the large amounts of new secretion which it was producing. The epithelium was cuboidal or low columnar in condition. The capillaries were enlarged and there was evidence of hypertrophy in the acinar walls.

Thus in the case of cats, an examination of the glands of pregnant females seems to show that, although on the whole the glands of pregnant females show a greater degree of activity than those of non-pregnant females, yet they vary in accordance with the seasonal changes recorded for normal females.

Only four glands were obtained from females whose mammary glands were actively secreting. These were obtained on October 27, November 8, April 20, and June 12 respectively. An examination of these glands showed that, like those of the pregnant females, each of them was more similar in condition to the glands of the normal females obtained at the same time, than to the glands of the other females in the lactation period during a different season of the year. For instance, the two glands obtained in October and November both contained very small acini and very little colloid (cf. normal females, p. 579). The earlier of the two was devoid of stainable colloid and seemed to consist merely of a mass of cells. The second gland contained colloid in some of its acini and was producing a certain amount of new secretion. The gland obtained on April 20 (fig. 9, Pl. 36),

like those obtained from normal females about the same date, contained much more colloid and the acini were altogether larger. The epithelium was on the whole cuboidal and contained masses of cells as a result of a previous condition of hypertrophy. Most of the large acini contained no new secretion, but there was a certain amount in some of the smaller ones. The fourth gland, obtained on June 12, contained acini in varying states of activity. There were a few very large ones with flat epithelium and no new secretion, but the major part of the gland was composed of smaller actively secreting acini with columnar epithelium and an abundance of new secretion. The colloid in the very large acini stained deeply with eosin, while that of the smaller acini was basophil. The connective tissue was somewhat thickened throughout the gland. The condition of this gland was somewhat similar to that observed in the glands of normal females about the same date (see p. 583).

Thus from the available data it appears that glands from females in the lactation period, like those of pregnant females, fall into line with the seasonal changes recorded for normal females.

SUMMARY OF FACTS REGARDING VARIATION IN CATS' THYROID.

Influence of Season.

1. Males.—From October–December the glands of male cats all contained large amounts of colloid, but were producing a certain amount of new secretion. Increased activity took place in January and February, but was replaced in March by a more passive condition. From June to August they were again actively secreting.

2. Females.—From October–December the females showed a slight degree of activity, but contained less colloid than the males. During the rest of the year the glands of females resembled those of males and seasonal changes affected both sexes equally.

Influence of Sex.

A sex difference was only apparent during the period October–

December. The facts for normal males are summarized above under 'Influence of Season'.

Only four castrated animals were examined.

Pregnancy and Lactation.

The glands of these females varied in accordance with the seasonal changes recorded for normal females. The glands of the pregnant females, however, were on the whole more actively secreting than those of normal females.

REFERENCES.

1. Bamber, R. C. (1924).—"An apparent connexion between Braxy and Thyroid activity", 'Nature', vol. 113, p. 161.
2. Fenger, F. (1914).—"Influence of castration and pregnancy on the iodine and phosphorus metabolism of the Thyroid", 'Journ. Biol. Chem.', vol. 17, p. 23.
3. Lowe, E. (1930).—"Variation in the histological condition of the thyroid glands of Sheep with regard to Season, Sex, Age, and Locality", 'Quart. Journ. Micr. Sci.', vol. 73, p. 437.
4. McCarrison, R. (1917).—"The Thyroid Gland in health and disease." London.
5. — (1925).—"Some problems of Thyroid disease", 'Brit. Med. Journ.', p. 1065.
6. Mills, C. A. (1918).—"Effects of external temperature, Morphine, Quinine, &c., on Thyroid activity", 'Amer. Journ. Physiol.', vol. 46, pp. 329-39.

EXPLANATION OF PLATES.

PLATE 35.

Fig. 1.—Part of longitudinal section of thyroid of female cat obtained on October 24, showing reduced amount of colloid and slight amount of new secretion.

Fig. 2.—Part of longitudinal section of gland of male cat obtained on October 28, showing larger amount of colloid than female and slight amount of new secretion.

Fig. 3.—Part of longitudinal section of gland of male cat obtained on January 27, showing actively secreting condition with a certain amount of hypertrophy.

Fig. 4.—Part of longitudinal section of gland of male cat obtained on February 18, showing active condition accompanied by hypertrophy.

Fig. 5.—Part of longitudinal section of gland of female cat obtained on same date as fig. 4, showing similar condition.

Fig. 6.—Part of longitudinal section of gland from pregnant female obtained on February 23, showing actively secreting condition.

PLATE 36.

Fig. 7.—Part of longitudinal section of gland from female obtained on March 10, showing colloid condition with no new secretion.

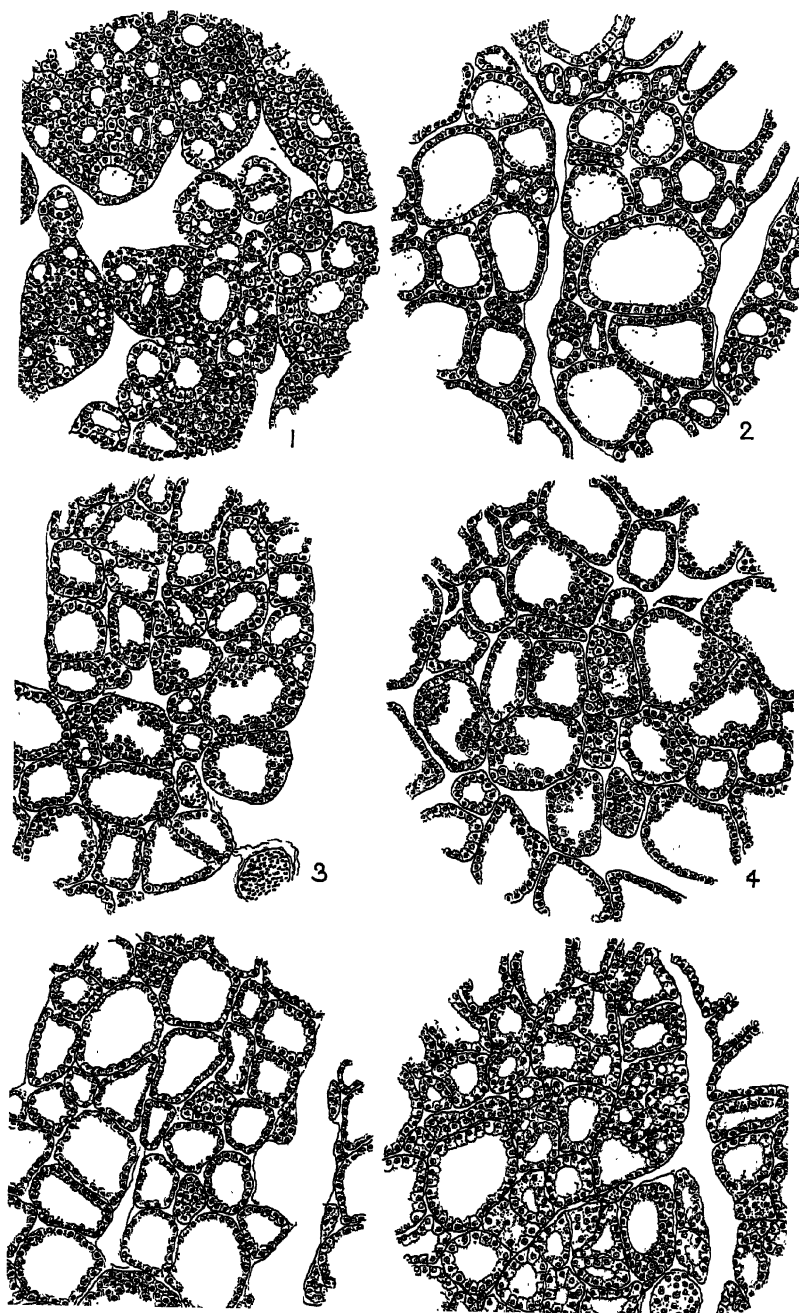
Fig. 8.—Part of longitudinal section of gland from male obtained on March 14, showing gland in colloid condition with flat epithelium and no new secretion. Cell-masses present from previous hypertrophy.

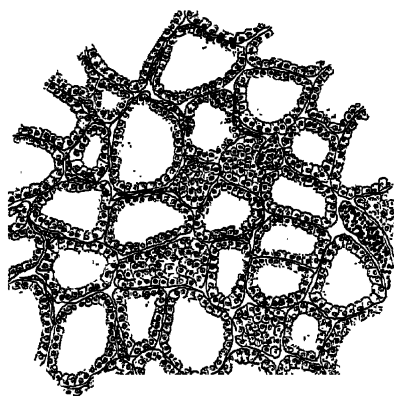
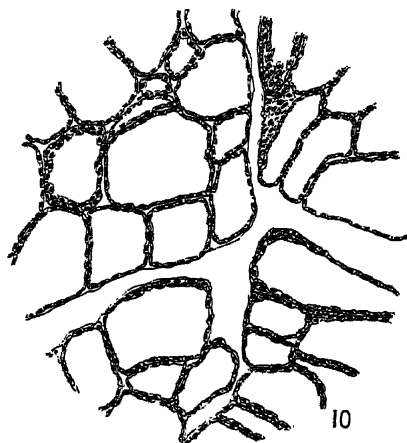
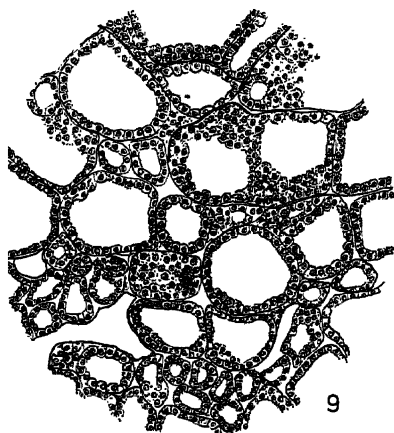
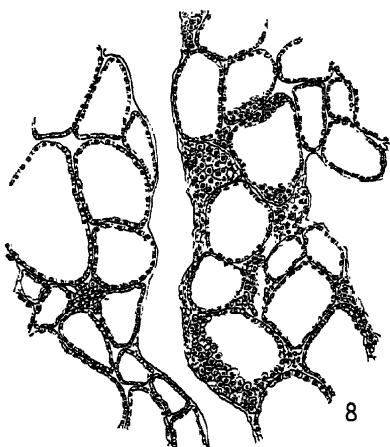
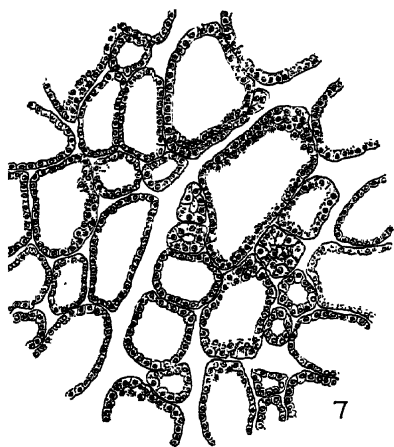
Fig. 9.—Part of longitudinal section of gland from female in lactation period obtained on April 20, showing no new secretion and containing cell-masses from previous hypertrophy.

Fig. 10.—Part of longitudinal section of gland from male cat obtained on same date as female (fig. 9), showing colloid condition with flat epithelium.

Fig. 11.—Part of longitudinal section of gland from male cat obtained on July 2, showing renewal of activity.

Fig. 12.—Part of longitudinal section of gland from female cat obtained on same date as male (fig. 11), showing similar condition.





The Formation of the Peritrophic Membrane in Insects, with Special Reference to the Larvae of Mosquitoes.

By

V. B. Wigglesworth, M.A., M.D.,

Lecturer and Milner Research Fellow in Medical Entomology at the London School of Hygiene and Tropical Medicine.

With 10 Text-figures.

CONTENTS.

	PAGE
1. INTRODUCTION	593
2. FORMATION OF THE PERITROPHIC MEMBRANE IN MOSQUITO LARVAE	594
(a) <i>Anopheles plumbeus</i>	595
(b) <i>Culex pipiens</i> and <i>Aedes (Stegomyia) argenteus</i>	598
3. FORMATION OF THE PERITROPHIC MEMBRANE IN OTHER DIPTERA	598
4. FORMATION OF THE PERITROPHIC MEMBRANE IN OTHER ORDERS OF INSECTS	601
(a) Hymenoptera	601
(b) Coleoptera	605
(c) Lepidoptera	605
(d) Aphaniptera	607
(e) Isoptera	608
(f) Neuroptera	608
(g) Odonata	609
(h) Orthoptera	611
(i) Dermaptera	612
5. DISCUSSION	612
6. SUMMARY	614
7. REFERENCES	615

1. INTRODUCTION.

In the larvae and adults of those Diptera in which a peritrophic membrane is present, it is a remarkably uniform and

discrete structure, and any theory of its formation must take this fact into account. Now it has recently been found (Wigglesworth, 1929) that in the tsetse-fly the peritrophic membrane is produced in the proventriculus as a fluid secretion, which is drawn through an annular cleft and so pressed and moulded to form a membranous tube. A similar origin was suggested by van Gehuchten (1890) for the membrane in the larva of *Ptychoptera* (Tipulidae); and Vignon (1901) showed that the proventriculus of *Chironomus* contains a beautiful delicate press in which the peritrophic membrane is milled. It is rather surprising that, although the anatomy of the intestine of various *Diptera* has been described, and the origin of the peritrophic membrane in them discussed, no attempt should have been made to discover cognate mechanisms by which the pressing of the membrane might be effected.

The present paper arose from an attempt to find such a mechanism in the larva of *Anopheles*. When this attempt proved successful the investigation was extended to the larvae of other *Nematocera*, and to the *Diptera* generally. Finally, it was found that analogous structures occur in most of the chief orders of insects. But before discussing briefly the varied forms which this mechanism may assume in other insects, a more detailed account will be given of the conditions present in the larvae of certain mosquitoes.

2. FORMATION OF THE PERITROPHIC MEMBRANE IN MOSQUITO LARVAE.

The majority of writers on the larvae of mosquitoes are agreed that the peritrophic membrane takes origin at the upper end of the mid-gut. At this point the fore-gut, the oesophagus, is invaginated into the mid-gut to form the structure usually referred to as the 'oesophageal valve', an unfortunate term that attributes to this part of the intestine a property which it does not possess; for the invagination acts not as a valve but as a sphincter. In the present paper the oesophageal valve together with the surrounding mid-gut will be referred to as the

proventriculus'.¹ The mid-gut of this region is conveniently called the 'cardia'.

The proventriculus of *Culex* and *Anopheles* larvae is accurately described by Thompson (1905). 'In the anterior part of the thorax the oesophagus dips into the cardia as the oesophageal valve . . . a deep curtain, thicker at the base than at the free border. At the free border the space between the inner or direct face and the outer or reflected face is occupied by a blood sinus. Above, the space is filled by the circular fibres of the annular muscle. . . . The oesophageal valve of *Anopheles* larva is very like that of *Culex*, but has a band of longitudinal muscles within the valve between the annular muscles and the epithelial cells of the upper part of the reflected face.' The description given by Imms (1907) agrees with this. In addition, he describes the peritrophic membrane as coming from the large, deeply staining cells of the cardia; but neither he nor Samtleben (1929) mention any mechanism by which this uniform membrane may be produced.

Such a mechanism has been sought in *Anopheles plumbeus*, *Culex pipiens*, and *Aedes* (*Stegomyia*) *argenteus*. A large number of larvae were rapidly killed by immersion in Carnoy's fixative; the proventriculus was dissected out, cleared, and mounted whole, either unstained or stained lightly with acid fuchsin. In this way the natural relations of the parts have been determined. The histological details have been elaborated from longitudinal sections. A striking thing about the proventriculus, when it is dissected out from all these species, is its rigidity. It maintains its cylindrical form when the other parts of the intestine readily collapse.

(a) *Anopheles plumbeus*.

In addition to the structures described by Thompson and by Imms, it has been found that in *Anopheles plumbeus* the longitudinal muscle of the oesophageal valve is inserted into the chitinous covering of the reflected face, and that at the point

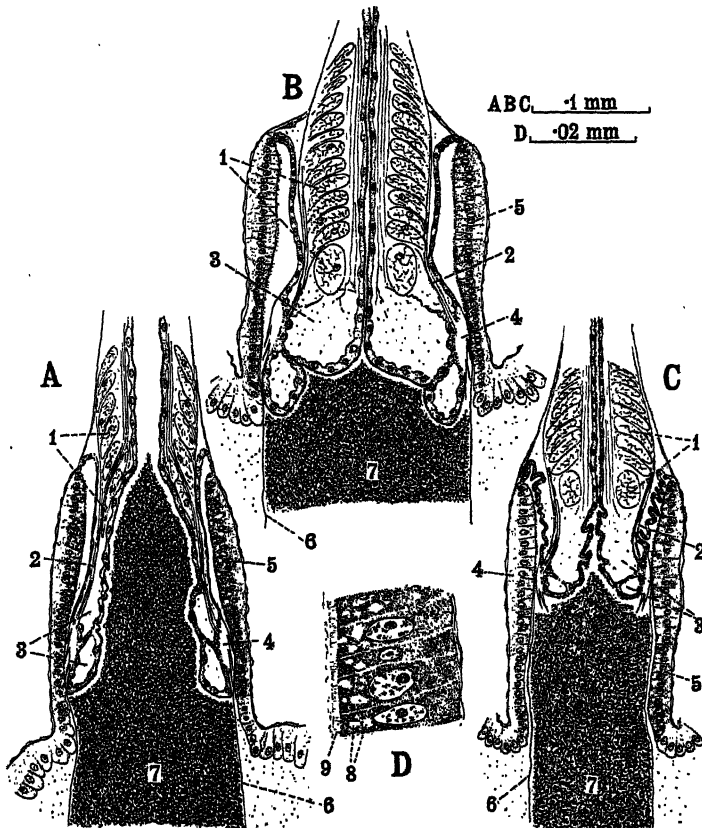
¹ This terminology may be used throughout the Diptera; but the 'proventriculus' in many insects (e.g. Hymenoptera) is a muscular organ belonging entirely to the fore-gut.

of insertion the chitin is greatly thickened to form a rigid ring ; a ring which is covered with delicate curved spines directed backwards and closely applied to the surface. In the normal position of relaxation (Text-fig. 1 A) this chitinous ring lies pressed against the lower end of the sheet of deeply staining cardiac cells which are regarded as the secreting cells of the peritrophic membrane. This sheet of cells has a somewhat conical form, tapering in front. It is clear, therefore, that when the longitudinal muscle contracts, the chitinous ring will be drawn forwards, firmly pressed against the secreting cells ; and in so doing the secretion which these discharge will be rolled out. When the muscle relaxes and the chitinous ring moves backwards again, the spines upon its surface will carry with them the newly formed sheet of peritrophic membrane. The rather delicate circular muscles outside the mid-gut will assist in keeping the parts in intimate contact. The circular muscle within the oesophageal valve will serve as a sphincter to close the upper part of the orifice; and when the sinuses in the free margin are distended they will serve to close the lower part.

Text-figs. 1 B and C show in action the various mechanisms deduced in the last paragraph from considerations of structure. In Text-fig. 1 B the circular muscles of the valve are firmly contracted and the blood sinuses tensely distended so as to occlude the lumen of the oesophagus. The longitudinal muscle is relaxed, and the chitinous ring lies at the hind end of the proventriculus. In Text-fig. 1 C the longitudinal muscle is contracted and the ring of chitin is drawn far forwards. The circular muscles also are contracted, but the sinuses are only partially distended.

The peritrophic membrane in mosquito larvae is not produced with such rapidity as that in the tsetse-fly (Wigglesworth, 1929), so that the secretory activity of the cells which produce it is proportionally less. But occasionally, in sections (Text-fig. 1 D), globules of secretion may be seen in process of extrusion from the cells. As in the case of the tsetse-fly again, since the membrane arises by the condensation of this fluid secretion into a solid sheet, it must necessarily fit the mould in which it is produced. This explains the well-known fact that the gut contents

TEXT-FIG. 1.



Proventriculus of *Anopheles plumbeus*. A, in normal position of relaxation; B, with sphincter muscle contracted and sinuses distended; C, with longitudinal muscle contracted; D, detail of cells secreting peritrophic membrane. 1, sphincter muscle; 2, longitudinal muscle; 3, blood sinuses; 4, chitinous thickening bearing spines; 5, cells of cardia secreting peritrophic membrane; 6, peritrophic membrane; 7, gut contents; 8, secretory vacuoles; 9, globules of secretion passing through striated border.

of the mosquito larva are always in the form of a cylindrical mass, equal in diameter with the oesophageal invagination and uniform throughout; whereas the wall of the intestine may show

considerable irregularities, including the large 'gastric caeca'. As in the tsetse-fly, the peritrophic membrane is composed of chitin. It resists solution in hot caustic alkalis, but after such treatment it gives the colour reactions for chitosan (Wester, 1910).

(b) *Culex pipiens* and *Aedes* (*Stegomyia*) *argenteus*.

In *Culex pipiens* the general mechanism is similar to that in *Anopheles*; but in place of a narrow ring near the free margin of the oesophageal valve, there is a general thickening of the chitin on the reflected surface. This surface has a double curve (Text-fig. 2 A), and at the free margin is provided with an everted rim. In short, the whole structure is more rigid than in *Anopheles*. The longitudinal muscle is absent, and no evidence could be obtained that the invagination moves up and down appreciably. No spines could be seen upon the chitinous thickening. On the other hand, the blood sinuses are well developed, and often, during closure of the orifice, they are fully distended (Text-fig. 2 B) and serve to occlude the lower end of the oesophagus, driving onwards the contents of the gut.

In *Aedes argenteus* (Text-fig. 2 C) the structure is almost identical with that in *Culex pipiens*. The chitinous covering of the free margin of the valve is, however, even more greatly thickened. It is worth noting that the characteristic form of this chitinous thickening is figured by Raschke (1887) in the larva of '*Culex nemorosus*' (= *Aedes* (*Ochlerotatus*) *nemorosus*), but it is not so clearly shown in the figures of subsequent authors.

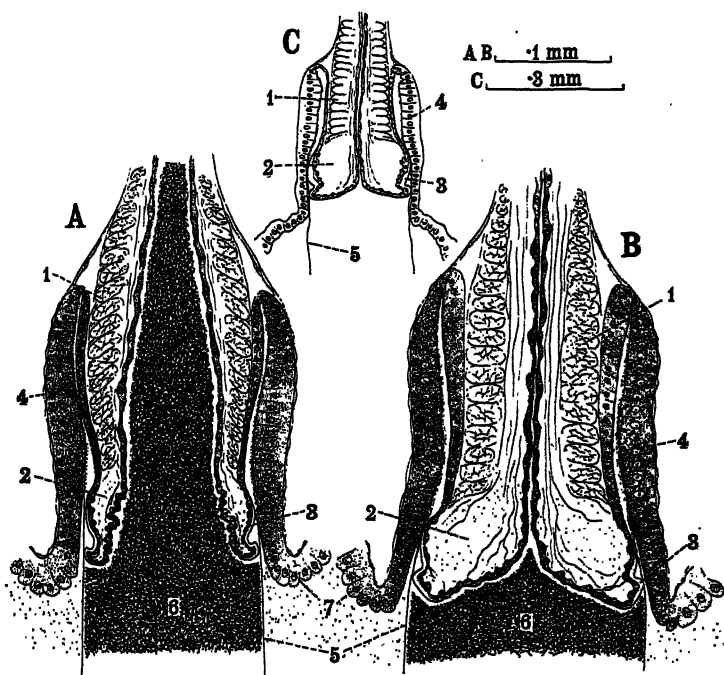
3. FORMATION OF THE PERITROPHIC MEMBRANE IN OTHER DIPTERA.

It is evident that the formation of the peritrophic membrane in the larvae of Culicidae is brought about in the same general way as that in the larvae of Ptychoptera and Chironomus. Indeed it seems probable, from an examination of the

literature, that the same is true for the larvae of all the Nematocera.

Thus in *Simulium* (Strickland, 1913) the 'oesophageal valve' contains a longitudinal muscle, as in *Anopheles*, and

TEXT-FIG. 2.

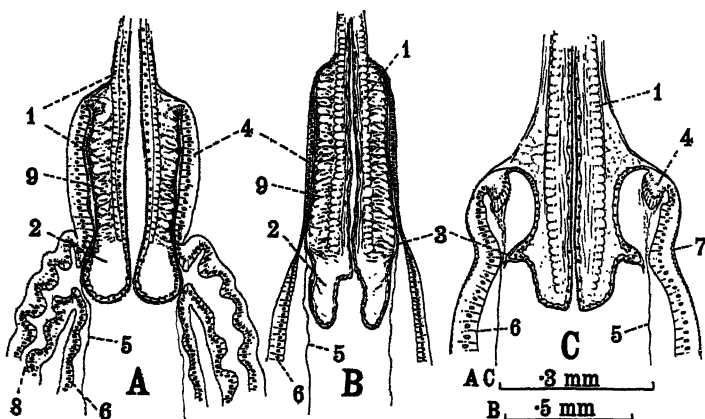


A, proventriculus of *Culex pipiens* in position of relaxation; B, the same with sphincter muscle contracted and sinuses distended; C, proventriculus of *Aedes argenteus*. 1, sphincter muscle; 2, blood sinuses; 3, chitinous thickening; 4, cells secreting peritrophic membrane; 5, peritrophic membrane; 6, gut contents; 7, wall of gastric caeca.

its free margin is covered with a thickened layer of chitin which bears rows of spines upon its surface. Strickland suggests that these spines serve to draw the peritrophic membrane backwards as it is formed, but he does not consider the possibility that the structure may act as a press. In *Forcipomyia* (*Ceratopogonidae*) Saunders (1924) figures the oesophageal valve and shows

a very thick layer of chitin on the outer surface, with a projecting flange near the free border. This doubtless has the same function. Wagner (1864) and Mecznirow (1866) figure the peritrophic membrane in *Miastor* (Cecidomyiidae) as a tube,

TEXT-FIG. 3.



Proventriculus of *Sciara* (A), *Rhyphus* (B), and *Telmatoscopus* (C). 1, sphincter muscle; 2, blood sinus; 3, chitinous thickening; 4, cells secreting peritrophic membrane; 5, peritrophic membrane; 6, wall of mid-gut; 7, circular muscle of 'press'; 8, gastric diverticula; 9, large vacuolated cells which fill the upper part of the 'oesophageal valve'.

equal in diameter with the oesophageal valve, uniform throughout its length, coiled freely within the dilated mid-intestine. In *Mycetobia* (Rhyphidae) Müller (1922) describes an annular thickening of the chitin of the outer wall of the valve, similar to that described above in *Anopheles*. He regards this structure as a mould ('Darminhaltspresse') in which the gut contents are compressed into little cylindrical masses. Almost certainly it has the same function as the chitinous ring in mosquito larvae.

I have myself examined the larvae of *Corethra* (Culicidae), *Rhyphus* (Rhyphidae), *Sciara* (Mycetophilidae), and *Telmatoscopus* (Psychodidae). In each of these there is a typical 'press' (Text-fig. 3 A, B, C) which it is unnecessary to describe in detail. The structure in *Telmatoscopus* is of particular

interest, for this is the only Nematocerous larva I have examined in which the arrangement resembles that described by Vignon (1901) in *Chironomus*, where the 'press' is entirely separated from the secretory cells.

Among the higher Diptera the same principle is encountered again. In the larva of *Calliphora* (figured by Perez, 1910) the 'oesophageal valve' is a solid cellular structure forming a plug which completely fills the proventriculus. There is no need for any thickening of the chitinous intima, the cells of the cardia which secrete the peritrophic membrane press directly upon this solid plug. The arrangement in the adult of *Drosophila* (figured by Chatton, 1920) is precisely the same and represents a condition intermediate between that present in the larvae of the Nematocera and in the adults of the Muscidae. In these the oesophageal invagination is everted funnel-wise and reflected over the enlarged epithelial cells which produce the membrane (Wigglesworth, 1929). One result of this change is that the diameter of the press is enlarged so that the diameter of the peritrophic membrane also is greatly increased.

4. FORMATION OF THE PERITROPHIC MEMBRANE IN OTHER ORDERS OF INSECTS.

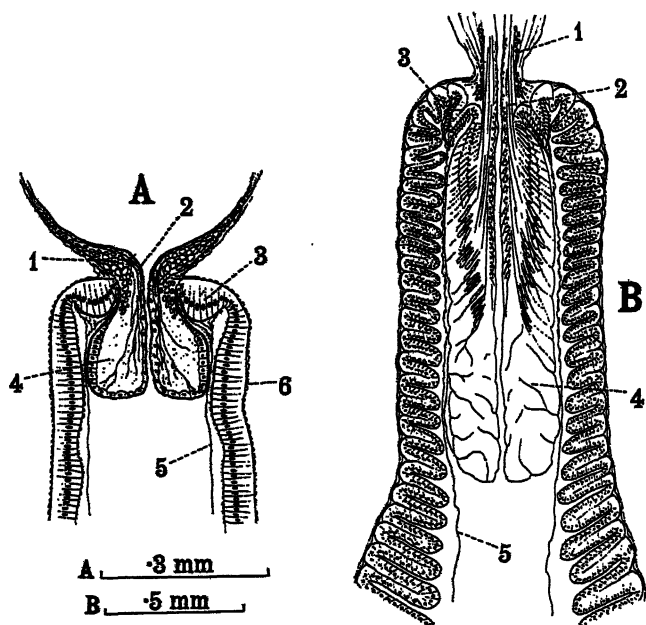
Where the peritrophic membrane is a strictly uniform tube, as it is in the Diptera, it is difficult to conceive any mechanism by which it could be formed unless this be in the nature of an annular mould or press. It was to be expected, therefore, that similar structures would be found in insects of other orders, and as will be shown in the account which follows, these have been observed in most of the main orders that have been examined.

(a) Hymenoptera.

In the larva of the wasp and bee, Anglas (1901) believed the peritrophic membrane to be secreted by large mid-gut cells which overlie the oesophageal valve, but no flattening mechanism was described. I have not examined these insects, but in a small saw-fly larva (*Tenthredinidae*) the peritrophic membrane arises from a ring of special cells in the cardia. Below this ring the oesophageal valve is pressed against the wall of the gut

either by the distension of the contained sinuses with fluid (Text-fig. 4 A), or by pressure of the gut contents as they pass through the valve. The membrane is composed of chitin.

TEXT-FIG. 4.



'Oesophageal valve' of saw-fly larva (A) and *Bombus* adult (B).
1, sphincter muscle; 2, longitudinal muscle; 3, cells secreting peritrophic membrane; 4, sinuses; 5, peritrophic membrane; 6, circular muscle of mid-gut.

In the adult of the bumble-bee (*Bombus*) Swingle (1927) states that the membrane is secreted by a collar of cells around the base of the oesophageal valve. The valve itself he figures in a collapsed state with folds of thin chitin trailing down the mid-gut; and this indeed is the usual appearance of the valve in mounted specimens; but if the gut of a freshly killed bumble-bee is dissected in normal saline, the valve will often be found tensely distended with fluid so that it is pressed against the wall of the mid-gut (Text-fig. 4 B) and the sleeve-like peritrophic membrane may be traced forwards over its surface to the ring

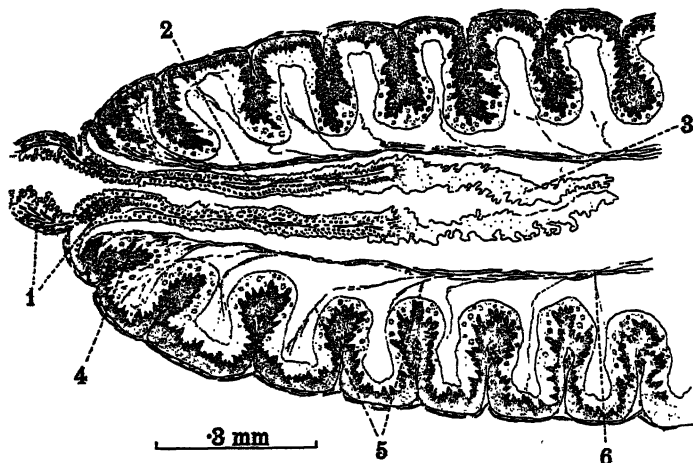
of cells by which it is produced. The precise mechanism by which the sinuses of the oesophageal valve, in this insect and others, are distended with fluid, is not clear; but the valve in the bee contains well-developed longitudinal and circular muscles at its base and these may play a part. In Text-fig. 4 *b* the membrane is shown as arising from the basal fold alone. It is uncertain whether the succeeding rings contribute to its formation; but if they do, this would account for the fact that in longitudinal sections the thickness of the membrane is seen to increase gradually up to the end of the oesophageal valve and then to remain constant. Furthermore, when the membrane is carefully dissected out in the fresh state it may sometimes be seen to be composed of several concentric layers. This membrane is chitinous.

In the honey-bee (*Apis*) Pavlovsky and Zarin (1922) and Snodgrass (1925) describe the peritrophic membrane as being produced by repeated delamination of the surface membrane of the mid-gut, brought about by the secretion pressure of the cells below. Certainly the membrane consists of a number of concentric layers, and between these layers are fragments of cytoplasm, often containing nuclei, which have clearly been shed off from the epithelium. It would be surprising, however, to find two such different modes of origin for the membrane in related forms with their anatomy so similar as the bumble-bee and the honey-bee. Moreover, the membrane gives the reactions of chitin, a fact which does not support the idea that it is shed off from the entire mid-gut.

A number of longitudinal sections of the mid-gut of the honey-bee have been examined. In these the appearance of the mid-gut cells varies considerably with the state of digestion; but in some sections they are precisely as figured by Pavlovsky and Zarin; overlaid by a number of delicate concentric membranes with cellular debris between, and it is impossible to doubt that these membranes are derived from the cells immediately beneath them. On the other hand, the main mass of the membrane always arises clearly from the anterior end of the mid-gut. Text-fig. 5 is typical of sections in this region. It will

be seen that the greater part of the membrane is streaming off the first fold of the mid-gut at the base of the oesophageal valve. Succeeding folds contribute to the membrane, but in diminishing degree, as the folds succeed each other down the gut.

TEXT-FIG. 5.



Longitudinal section of anterior end of mid-gut in *Apis*. 1, sphincter muscle; 2, cells of 'oesophageal valve'; 3, blood sinus in collapsed state; 4, basal fold of mid-gut, from which the greater part of the peritrophic membrane is arising; 5, smaller contributions to the membrane by succeeding folds; 6, peritrophic membrane.

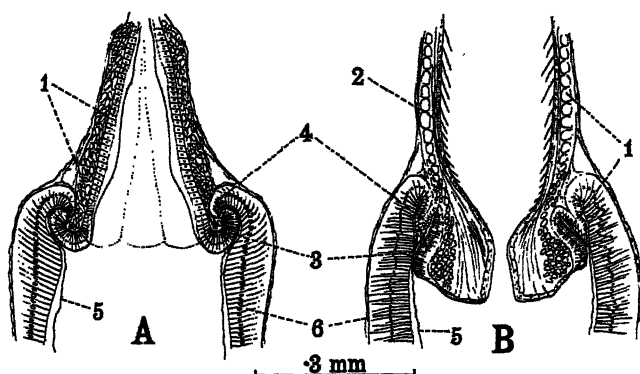
It must, therefore, be concluded that the peritrophic membrane of the honey-bee is of two-fold origin; a chitinous basis, itself composed probably of several concentric tubes, secreted at the anterior end of the mid-gut; and a series of indefinite membranes which condense upon the outside of this as it proceeds down the gut.¹ The pressing of the basic membrane is probably brought about in the same way as in *Bombus*; but the efficiency of this press, at least in the honey-bee, is not so great as of those described in the *Diptera*, and the membrane is not such a clean-cut and uniform tube.

¹ I have not actually demonstrated that the inner layers are chitinous and the outer layers not chitinous. F. L. Campbell (1929), *Ann. Ent. Soc. Amer.*, xxii., has also shown the presence of chitin in the peritrophic membrane of the bee.

(b) Coleoptera.

A peritrophic membrane is present in many Coleoptera, though it is said to be absent in certain of the carnivorous groups (Carabidae, Dytiscidae). For the purpose of this paper it has been studied in the larva of *Tenebrio molitor* and the adult of *Coccinella septempunctata*. In both of these

TEXT-FIG. 6.



'Oesophageal valve' of young larva of *Tenebrio molitor* (A) and *Coccinella* adult (B). 1, sphincter muscle; 2, longitudinal muscle; 3, rigid ring of chitin-covered cells forming the inner surface of the 'press'; 4, cells secreting peritrophic membrane; 5, peritrophic membrane; 6, wall of mid-gut.

a press is present (Text-fig. 6 A, B) and in both of them the membrane is chitinous.

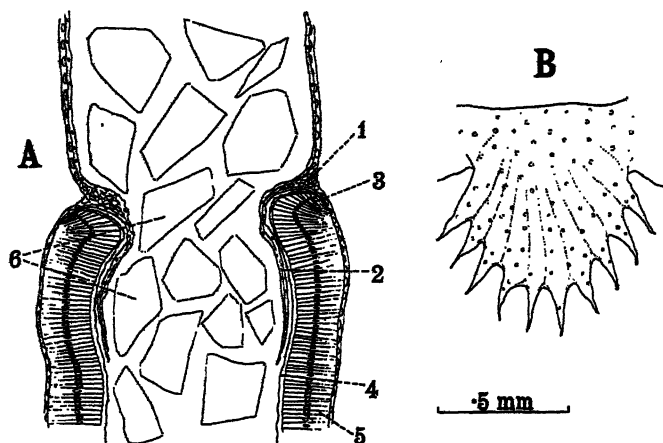
(c) Lepidoptera.

Vignon (1901) examined the oesophageal valve of the silk-worm. He could not detect any mechanism for flattening the peritrophic membrane and concluded that this was formed by the condensation of secretion from the general epithelium of the mid-gut upon the surface of the gut contents; and associated with this mode of origin he noted that the thickness of the membrane varied greatly in different parts. Bordas (1911) studied the intestine in a great many lepidopterous larvae and found in every case that the peritrophic membrane was secreted by a ring of deeply staining granular cells around the base of

the oesophageal valve. No mechanism for pressing the membrane was described.

The question has been re-investigated in the larva of *Cheimabacche fagella* (Oecophoridae), *Ephestia kuhniella* (Pyralidae), and *Sitotroga cerealella* (Gelechiadae). In

TEXT-FIG. 7.



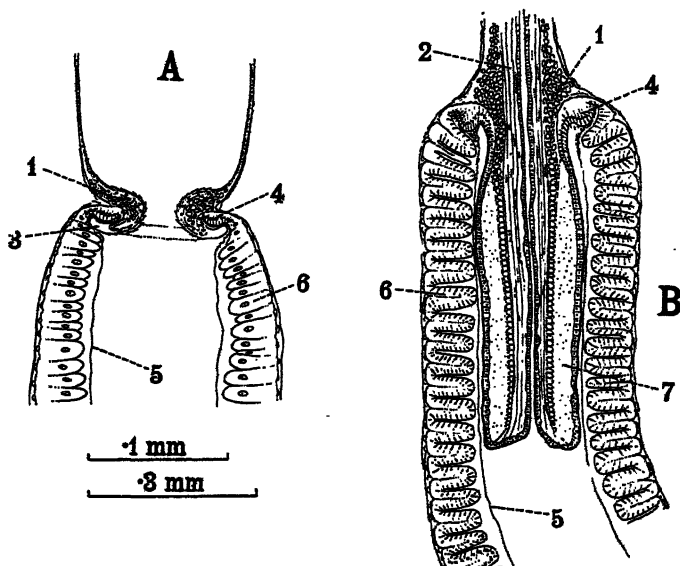
A, 'oesophageal valve' of lepidopterous larva (*Cheimabacche fagella*). 1, sphincter muscle; 2, invaginated portion of fore-gut; 3, cells secreting peritrophic membrane; 4, peritrophic membrane; 5, mid-gut; 6, gut contents. B, surface view of one of the three delicate leaflets which compose the invaginated portion of the fore-gut (2).

all these the oesophageal valve hangs down into the mid-gut as a most delicate curtain made up of several overlapping leaflets which, in *Cheimabacche fagella*, are three in number and are regularly fringed at the lower border (Text-fig. 7 B). In the normal state of the larva the gut is always firmly distended, and this chitinous curtain is pressed against the epithelium of the mid-gut and not drawn inwards as it would be were it acting as a valve (Bordas, 1911).¹ So long, therefore, as the gut is

¹ The incompetence of this structure to serve as a valve may be demonstrated by removing the head of a freshly killed larva. The contents of the mid-gut are instantly driven forwards into the fore-gut, often carrying with them the peritrophic membrane and causing retroversion of the 'oesophageal valve'.

distended, the 'oesophageal valve' will constitute a more or less efficient press for the peritrophic membrane, which extends forwards beneath it to the anterior limit of the mid-gut (Text-fig. 7 A). In those larvae in which the valve contains blood sinuses (as described by Bordas in *Lo irene*) these may perhaps become distended and press upon the membrane as observed in

TEXT-FIG. 8.



'Oesophageal valve' of flea larva (*Ceratophyllus wickhami*) (A) and of termite (B). 1, sphincter muscle; 2, longitudinal muscle; 3, chitinous ring forming inner surface of 'press'; 4, cells secreting peritrophic membrane; 5, peritrophic membrane; 6, mid-gut; 7, blood sinus.

the tenthredinid larva (Text-fig. 4 A). As in the other insects, the membrane in the lepidopterous larva is chitinous.

(d) *Aphaniptera*.

In the larva of *Ceratophyllus wickhami* (Text-fig. 8 A), the oesophageal invagination does not bear even a superficial resemblance to a valve. The margins of the invagination are composed of thickened chitin and are reflected over and

closely applied to the cells which secrete the peritrophic membrane.

(e) Isoptera.

The general appearance of the crop, the proventriculus and the upper end of the mid-gut in the termite is strikingly similar to that in the bumble-bee, and the similarity extends to the mode of origin of the chitinous peritrophic membrane (Text-fig. 8 B). It should be noted that the base of the oesophageal valve is surrounded by an annular fold which secretes the membrane, but that the succeeding projections from the wall of the gut are villi and not complete circular folds as they are in *Bombus*. The oesophageal valve is a hollow vascular structure as in the bee, and again the pressure is probably effected by its distension with fluid.

(f) Neuroptera.

The alimentary tract in the larva and adult of *Chrysopa* was studied by McDunnough (1909). He considered that in the larva the peritrophic membrane was secreted by the general epithelium of the mid-gut. In the adult he observed that the membrane was attached at the anterior end of the mid-gut to a ring of deeply staining columnar cells around the base of the oesophageal valve. He did not, however, regard these cells as the source of the membrane, but believed that it arose by separation of the surface-layer of the epithelial cells of the mid-gut, and that this separation failed to occur in the neighbourhood of the oesophageal valve.

A few larvae of *Hemerobius* have been examined. The peritrophic membrane was found to be composed of chitin, but its mode of origin was not determined. As shown by McDunnough in *Chrysopa*, the oesophageal valve in these larvae is very much reduced. In a single adult of *Hemerobius* the structure of the oesophageal invagination was precisely similar to that figured by McDunnough in *Chrysopa*. In the living state the sinuses in the invagination were firmly distended and in contact with the wall of the gut below the ring of

cells to which the peritrophic membrane was attached. There seems no reason to doubt that the manner of production of the membrane is the same here as in the other insects described.

(g) Odonata.

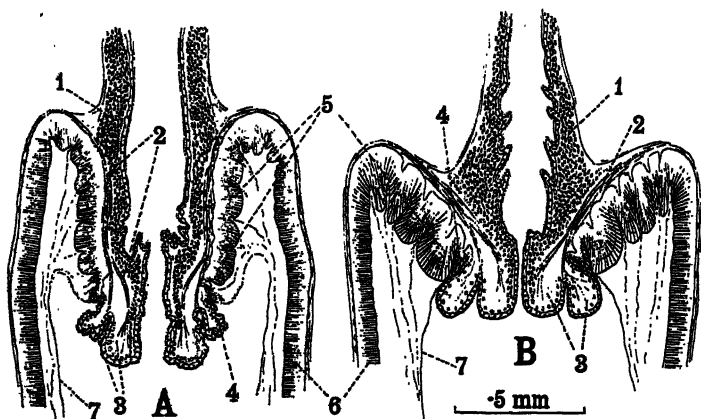
The formation of the peritrophic membrane in the larva of *Aeschna* was carefully studied by Voinov (1898); and since his conclusions are at variance with those recorded in this paper it is necessary to consider the question in some detail. Voinov observed a ring of deeply staining columnar cells at the point of junction of the fore-gut and mid-gut in the larva of *Aeschna*, and he noted that the peritrophic membrane was attached to this ring of cells. He did not believe, however, that they were responsible for its formation. He considered the membrane to be the striated border of the cells of the mid-gut separated by the globules of secretion beneath; and stated that, in sections, the peritrophic membrane may be seen in places to become continuous with the striated border. Not having the conception of an annular press, he points out with perfect justice that the production of a membrane by a simple ring of cells is 'incomprehensible'.

Larvae of *Aeschna* have been studied by dissection, by clearing and mounting the gut entire, and by microscopic sections. The usual appearance of the gut in longitudinal section is seen in Text-fig. 9 A, which shows the extensive invagination of the fore-gut and the attachment of the peritrophic membrane near the free border of this invagination. No pressing mechanism can be seen. This, however, is the appearance in the fasting larva; whereas the production of the peritrophic membrane will be most active after a meal. A number of larvae were therefore given a copious feed of *Lucilia* maggots and examined at varying intervals thereafter. Many of the larvae in this series showed the appearance indicated in Text-fig. 9 B. The invagination of the fore-gut was not so extreme, and the ring of deeply staining cells now lay nearer the front end of the mid-gut. The circular sinuses in the free

margin of the invagination were more or less distended, coming in contact with the wall of the mid-gut beyond the ring of cells, and forming a press for the secretion of these cells, which issued from the cleft as the peritrophic membrane.

There seems little doubt that the mode of formation described in the other insects in this paper obtains also in the larva of *Aeschna*; and here again the membrane is composed of

TEXT-FIG. 9.



'Oesophageal valve' of the larva of *Aeschna*. A, in position of relaxation; B, drawn forwards, with sinuses distended. 1, sphincter muscle; 2, longitudinal muscle; 3, blood sinuses; 4, chief cells secreting peritrophic membrane; 5, cells which contribute to the membrane; 6, wall of mid-gut; 7, peritrophic membrane composed of several layers.

chitin. On the other hand, the histological changes in the mid-gut as figured by Voinov undoubtedly occur, and, at certain stages of digestion, indefinite membranes formed from the mid-gut secretion separate from the cells and condense upon the contents of the gut. But these membranes are merely added to the outside of the chitinous tube which is the peritrophic membrane proper.¹ A further complication arises from the fact that the cells of the mid-gut adjacent to the initial ring also contribute to the membrane (see Text-fig. 9 B). The result is

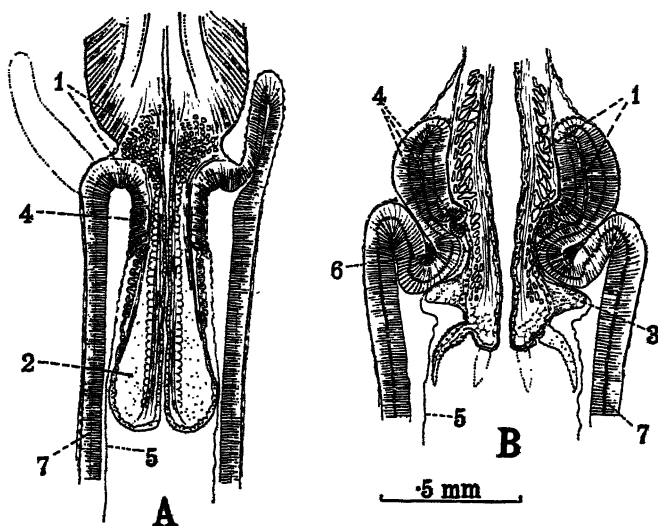
¹ See foot-note, p. 604.

that the peritrophic membrane in this insect, as ordinarily observed, is a somewhat indefinite structure derived from a number of sources.

(h) Orthoptera.

Cuénot (1895) described the peritrophic membrane in *Peri-*

TEXT-FIG. 10.



'Oesophageal valve' of *Blatella germanica* (A) and *Forficula* (B). 1, sphincter muscle; 2, blood sinus; 3, rigid ring of chitin; 4, cells secreting peritrophic membrane; 5, peritrophic membrane; 6, circular muscle actuating the 'press'; 7, epithelium of mid-gut.

planeta and *Ectobia* as being secreted by a ring of special cells at the base of the oesophageal valve.

In *Blatella germanica* (Text-fig. 10 A) the arrangement is similar to that figured by Cuénot in *Ectobia*. The oesophageal valve is long and thin-walled, and, in freshly killed insects dissected in normal saline, is often found to be distended with fluid, so forming an efficient press for the chitinous peritrophic membrane.

(i) *Dermaptera*.

Cuénot (1895) figured the peritrophic membrane in the earwig (*Forficula*) as derived from special cells as in the *Orthoptera*, but he did not describe any flattening mechanism. On re-examining this insect, however, it was apparent at once that the secretory cells in question are large and extensive, and that their secretion passes through a very rigid and efficient press (Text-fig. 10 B). It is not surprising, therefore, that the peritrophic membrane in the earwig should be a very well-defined and tough chitinous tube.

5. DISCUSSION.

It is the current opinion of most writers upon the peritrophic membrane of insects that this is a different morphological structure in different orders; an opinion supported mainly by the observations on the honey-bee (*Apis*) and on the larva of the dragon-fly (*Aeschna*), which have already been discussed. The observations recorded in the present paper do not entirely refute this view; but they serve to show, on the one hand, that wherever a peritrophic membrane is present it is composed of chitin, and, on the other, that an annular press is very frequently responsible for its formation. The work is not sufficiently extensive to warrant any generalization, but it does suggest that the so-called 'oesophageal valve' of insects, which, under the non-committal term of 'Rüssel', Schneider (1889) showed to be of such widespread occurrence, never functions as a valve at all, but always as a sphincter between the fore-gut and the mid-gut, and frequently as a press for the peritrophic membrane.

The mechanical efficiency of this press varies greatly from one order to another; and the mechanism attains its most specialized forms in the *Diptera*. It is not surprising that the perfection of the peritrophic membrane should run parallel with the refinement of the press in which it is milled. Where the press is best developed the cells which produce the membrane are most clearly differentiated. Where the press is less efficient (as is well seen in *Apis* and in the larva of *Aeschna*) the

specialization of certain cells for the production of the membrane is incomplete ; and although the greater part of the substance is derived from the anterior end of the mid-gut, the cells further back contribute to it to some extent. This suggests the possibility that in yet other insects (or other Arthropods) the production of the membrane may be more generalized still, and distributed throughout the length of the mid-gut.¹

With regard to the composition of the peritrophic membrane, it is interesting to recall certain observations by Wester (1910). On examining the mid-gut of various arthropods he noted that this was completely lined with chitin in *Periplaneta* and *Melolontha*, though not in *Dytiscus*. In *Scolopendra* there was chitin in one specimen but not in another. Wester does not appear to have been familiar with the peritrophic membrane, but his results may be readily understood in the light of what is known of the distribution of that structure.

As to the function of the peritrophic membrane, no direct evidence can be adduced. It is present in the majority of insects, and its distribution is generally said to follow fairly closely the nature of their food. It is present, for example, in the more primitive orders, but has been lost in the Hemiptera and in adult Lepidoptera, insects which feed only upon fluids, and in the Carabidae and Dytiscidae among Coleoptera, in which digestion is extra-intestinal. It is, therefore, said to be 'protective' to the epithelium. No such structure, however, is present in the alimentary canal of vertebrates ; but there the entire tract is furnished with mucous glands, which provide the epithelium with a protective coating of mucin, lubricating the solid masses in the gut. Mucous glands are entirely wanting in the alimentary tract of insects,² and it is possible that their

¹ Balbiani (1890) states that in *Cryptops* (Myriapoda), in which oesophageal invagination is wanting, the peritrophic membrane is a product of the general epithelium of the mid-gut.

² The 'goblet cells' recently described by Henson (1929) in the larva of *Vanessa urticae* (Lepidoptera), and which contain material 'optically indistinguishable from striated border', appear to be of a different nature from the mucous glands of the vertebrate gut.

function is served by the peritrophic membrane. It has been shown in the case of the tsetse-fly to be freely permeable to the digestive enzymes and to the products of digestion (Wigglesworth, 1929).

6. SUMMARY.

In the larvae of mosquitoes (*Anopheles*, *Culex*; and *Aedes*) the secretion from the cells of the cardia, in the pro-ventriculus, is drawn through an annular press and thereby moulded to form the peritrophic membrane. The mechanism of this press has been described in detail.

It seems probable that, throughout the Diptera, the peritrophic membrane is formed by similar mechanisms. Figures are given of those in the larvae of *Sciara* (Cecidomyiidae), *Rhyphus* (Rhyphidae), and *Telmatoscopus* (Psychodidae).

Analogous structures (a zone of secreting cells in connexion with an annular press) have been found in most of the main orders of insects, as follows: Hymenoptera [adult of *Bombus* and *Apis* and the larva of a saw-fly (Tenthredinidae)]; Coleoptera [larva of the mealworm (*Tenebrio molitor*) and the adult of *Coccinella*]; Lepidoptera [larvae of *Cheimabacche fagella* (Oecophoridae), *Sitotroga cerealella* (Gelechiidae) and *Ephestia kuhniella* (Pyralidae)]; Aphaniptera (larva of *Ceratophyllus wickhami*); Isoptera; Neuroptera (adult of *Hemerobius*); Odonata (larva of *Aeschna*); Orthoptera (*Blatella germanica*); and Dermaptera.

In every case, in addition to its function as a press, the so-called 'oesophageal valve' was found to act not as a valve but as a sphincter.

In the honey-bee (*Apis*), the larva of the dragon-fly (*Aeschna*), and possibly in other insects, indefinite membranes are shed off by the cells farther back in the mid-gut, and added to those produced in the annular press.

In all the insects examined, chitin formed the basis of the peritrophic membrane.

The observations recorded give a coherent significance to much of the previous work on the subject, which, before, appeared contradictory.

7. REFERENCES.

- Anglas, M. J. (1901).—"Observations sur les métamorphoses internes de la guêpe et de l'abeille", 'Bull. Sci. Fr. et Belg.', xxxiv.
- Balbani, E. G. (1890).—"Études anatomiques et histologiques sur le tube digestif des Cryptops", 'Arch. Zool. Exp. et Gén.', viii.
- Bordas, L. (1911).—"L'appareil digestif et les tubes de Malpighi des larves des Lépidoptères", 'Ann. Sci. Nat.', ix.
- Chatton, E. (1920).—"Les membranes péritrophiques des Drosophiles (Diptères) et des Daphnies (Cladocères); leur genèse et leur rôle à l'égard des parasites intestinaux", 'Bull. Soc. Zool. de France', xlv.
- Cuénot, L. (1895).—"Études physiologiques sur les Orthoptères", 'Arch. de Biol.', xiv.
- van Gehuchten, A. (1890).—"Recherches histologiques sur l'appareil digestif de la larve de la *Ptychoptera contaminata*", 'La Cellule', vi.
- Henson, H. (1929).—"On the development of the mid-gut in the larval stages of *Vanessa urticae* (Lepidoptera)", 'Quart. Journ. Micr. Sci.', lxxiii.
- Imms, A. D. (1907).—"On the larval and pupal stages of *Anopheles maculipennis*, Mg.", 'Journ. Hyg.', vii.
- McDunnough, J. (1909).—"Über den Bau des Darms und seiner Anhänge von *Chrysops perla*, L.", 'Arch. Naturg.'
- Mecznikow, E. (1866).—"Embryologische Studien an Insecten", 'Zeit. Wiss. Zool.', xvi.
- Müller, M. (1922).—"Rhyphus und Mycetobia mit besonderer Berücksichtigung des larvalen Darms", 'Arch. Naturg.', lxxxviii.
- Pavlovsky, E. N., and Zarin, E. J. (1922).—"On the structure of the alimentary canal and its ferments in the Bee (*Apis mellifera*, L.)", 'Quart. Journ. Micr. Sci.', lxvi.
- Perez, C. (1910).—"Recherches histologiques sur la métamorphose des muscides (*Calliphora erythrocephala*, Mg.)", 'Arch. Zool. Exp. et Gén.', iv.
- Raschke, E. W. (1887).—"Die Larve von *Culex nemorosus*", 'Arch. Naturg.', liii.
- Samtleben, B. (1929).—"Zur Kenntnis der Histologie und Metamorphose des Mitteldarms der Stechmückenlarven", 'Zool. Anz.', lxxxi.
- Saunders, L. G. (1924).—"On the life-history and the anatomy of the early stages of *Forcipomyia* (Diptera. Nemat. Ceratopogoninae)", 'Parasitology', xvi.

- Strickland, E. H. (1913).—"Further observations on the parasites of *Simulium* larvae", 'Journ. Morph.', xxiv.
- Snodgrass, R. E. (1925).—"Anatomy and Physiology of the Honey-bee." New York.
- Swingle, M. C. (1927).—"The alimentary tract of the common bumble-bee", 'Ohio Journ. Sci.', xxvii.
- Thompson, M. T. (1905).—"Alimentary canal of the mosquito", 'Proc. Boston Soc. Nat. Hist.', xxxii.
- Vignon, P. (1901).—"Recherches de cytologie générale sur les épithéliums", 'Arch. de Zool. Exp. et Gén.', ix.
- Voinov, D. N. (1898).—"Recherches physiologiques sur l'appareil digestif et le tissu adipeux des larves des Odonates", 'Bull. Soc. Sci. Bucharest', vii.
- Wagner, N. (1863).—"Beitrag zur Lehre von der Fortpflanzung der Insectenlarven", 'Zeit. Wiss. Zool.', xiii.
- Wester, D. H. (1910).—"Über die Verbreitung u. Lokalisation des Chitins im Tierreiche", 'Zool. Jahrb. Abt. Syst.', xxviii.
- Wigglesworth, V. B. (1929).—"Digestion in the tsetse-fly: a study of structure and function", 'Parasitology', xxi.

Certain Phenomena of Tenthredinid Oogenesis as revealed mainly by Feulgen's Nuclear-Reaction.

By

R. A. R. Gresson, B.Sc.,

From the Department of Natural History, University College (University
of St. Andrews), Dundee.

With Plate 37 and 4 Text-figures.

CONTENTS.

	PAGE
I. INTRODUCTION	617
II. PREVIOUS WORK	618
III. MATERIAL AND METHODS	620
IV. OBSERVATIONS	621
1. Nurse-cells	621
2. Follicle-cells	622
3. Oocytes	625
V. DISCUSSION	625
VI. SUMMARY	628
VII. CONCLUSIONS	629
VIII. ACKNOWLEDGEMENTS	630
IX. REFERENCES	630
EXPLANATION OF PLATE	631

I. INTRODUCTION.

In two recent papers on saw-fly oogenesis the writer (4 and 5) has shown that, in the three species investigated, the oxyphil and basophil nucleoli give rise to buds which are extruded into the ooplasm; and in a former contribution (Peacock and Gresson, 9) certain points relating to the behaviour of the nurse-cells and follicle-cells have been described. By means of Feulgen's technique further light has been thrown on certain of the phenomena already recorded.

II. PREVIOUS WORK.

Feulgen's 'Nuclealreaktion' (3) is based on Schiff's test for aldehydes and is considered a specific stain for chromatin. Thus Ludford (8) states that 'the general consensus of opinion is that the method provides a precise microchemical colour reaction for thymusnucleic acid protein complexes. No other cell-structures, or inclusions of any kind are stained, when the correct procedure is employed.'

Koch (7) has used this method for the study of the oogenesis of *Chilopods*. Chromatin was demonstrated in the oogonia and follicle-cells, but was not observed in the oocytes; he concludes that the chromatin undergoes a profound chemical change during the growth of the oocyte.

During the oogenesis of *Limnaea stagnalis* Ludford (8) states that 'scarcely any chromatin is distinguishable in the nucleus by Feulgen's method'. Some of the nuclei showed a 'faint purple colouration', so that he is 'inclined to believe that the chromatin is so finely dispersed in the nucleus as to render its demonstration impossible by this method'.

It may be, however, as Koch has suggested, that during oögenesis the chromatin undergoes profound chemical changes. Ludford could not detect chromatin in either the oxyphil or basophil staining nucleoli, but the nurse-cells contained a large amount.

In the young oocyte of the mouse Ludford (8) finds that the nucleus contains, in addition to small granules of chromatin, 'a considerable amount of material which stains with light green'. The nucleolus does not contain chromatin, 'although small granules frequently lie in contact with it'. The chromatin does not appreciably increase in amount during the growth of the oocyte but the non-chromatinic material does.

In a former contribution (Peacock and Gresson, 9) the nurse-cell nuclei of *Thrinax mixta* and *Allantus pallipes* (as well as those of certain other species not dealt with in the present work) were described as being covered by a cloud of darkly staining granules of chromatin, while the appearances of

the nuclear material indicated that the nuclei were undergoing a period of activity. Thus in the early nurse-cells of *Allantus pallipes* the nuclear material is aggregated into a compact mass, while in the older cells these masses become fragmented and distributed in the nuclei. It was concluded that the chromatin granules in the cytoplasm originated from the nurse-cell nuclei and finally gave origin to accessory nuclei. It should be pointed out that as the *Allantus pallipes* material was treated by Bensley's method, while the *Thrinax mixta* ovaries were fixed in Bouin's fixative and subsequently stained in iron haematoxylin, it was highly desirable that the phenomena be reinvestigated with the aid of a specific stain for chromatin.

In *Thrinax mixta* some of the follicle-cells revealed the presence of darkly stained granular material probably originating from the nucleus. Later the granules pass out of the follicle-cells into the oocyte, where, it was thought, they may give rise to accessory nuclei.

In the same paper it was stated that the nucleolar buds gave origin to accessory nuclei. This, however, has subsequently been shown by the writer not to be the case in *Thrinax macula*, *Allantus pallipes* (4), and *Thrinax mixta* (5).

In *Thrinax macula* an oxyphil nucleolus becomes separated from the original basophil nucleolus of the early oocytes and in *Thrinax mixta* an oxyphil nucleolus appears close to the basophil nucleolus of the oocyte; later both types of nucleoli give rise to buds which are extruded to the ooplasm. In *Allantus pallipes* the early basophil nucleolus changes as a whole from basophil to oxyphil, the basophil material being represented by spherical bodies with basophil granules residual after the transformation of the original nucleolus.

The *Thrinax mixta* material showed clearly that the basophil extrusions pass through the nuclear membrane and become dissolved in the ooplasm; the fate of the oxyphil extrusions is probably closely similar. In the present contribution *Thrinax mixta* and *Allantus pallipes* material is reinvestigated in order to determine if chromatin granules

are passed from the nurse-cell nuclei into the cytoplasm as well as extruded into the ooplasm from the follicle cell nuclei or with the nucleolar extrusions.

To summarize: granules described as chromatin occur round the nurse-cell nuclei of certain saw-flies; they originate from the nuclei. In *Thrinax mixta* darkly stained granules are passed from the follicle-cells to the oocytes; both types of emissions give origin to accessory nuclei. In *Thrinax mixta*, *Thrinax macula*, and *Allantus pallipes oxyphil* and basophil nucleolar extrusions occur; these become dissolved in the ooplasm. Koch, for Chilopods (7), believes that during the growth of the oocyte the chromatin undergoes a chemical change. In *Limnaea stagnalis* Ludford (8) is inclined to believe that during oogenesis the chromatin is so finely dispersed as to render its demonstration by Feulgen's method impossible; he could not detect chromatin in either the oxyphil or basophil nucleoli. In the mouse (op. cit.) the nucleolus does not contain chromatin; the chromatin does not appreciably increase in amount during the growth of the oocyte.

III. MATERIAL AND METHODS.

The material for this paper was obtained from specimens of *Thrinax mixta* Kl. and *Allantus (Emphytus) pallipes* Spin. (Enslin, 2), the former species in April 1929 from pupae which had hibernated in the larval condition, the latter from corresponding stages in June 1929.

In all cases the following procedure was adopted; the ovaries were dissected out in saline solution and immediately fixed in corrosive acetic fixative; sections were cut 5μ in thickness, subsequently treated by Feulgen's method¹ and counter stained in light green.

¹ Stain prepared from Grubler's basic fuchsin gave the Feulgen reaction, while that prepared from another source did not do so, and, in fact, caused great trouble and loss of time. This observation is supported by Miss M. A. M. Fullegar, B.Sc., of this department, who very kindly paralleled certain of my tests with my material and also with her own research material consisting of mammalian spleen, Oligochaetes and Sporozoans.

The above material was checked against ovaries fixed in corrosive acetic fixative and stained in Mann's methyl-blue eosin.

IV. OBSERVATIONS.

1. Nurse-cells.

Certain of the *Allantus pallipes* material proved to be less advanced in development than the other ovaries of the series. In this material the chromatin of the early undifferentiated nurse-cells at the proximal end of the ovarioles, as revealed by Feulgen's 'nuclealreaktion', exist in the form of large granules, the majority of which occur close to the nuclear membrane (fig. 1, Pl. 37). With the growth of the cells a nuclear network (which gives the chromatin reaction) makes its appearance, and in it small chromatin granules are distributed; the large granules of the younger cells are no longer present (fig. 2, Pl. 37). The exact structure of the nuclear network is difficult to determine, as it is revealed as an indefinite background to the granules. After treatment by Feulgen's method both chromatin granules and network take on a purplish colouration. In the nurse-cells situated in the fully formed nutritive chambers towards the posterior end of the ovarioles, the network extends throughout the entire nucleus leaving only small spaces, free of chromatin, between its meshes; the chromatin granules, as in the earlier cells, are distributed in the network (fig. 7, Pl. 37). In *Thrinax mixta* the ovarioles were more highly developed and consequently the youngest cells were more mature than the earliest nurse-cells described for *Allantus pallipes*. In the former species the chromatin of the nurse-cells exists in the form of granules distributed throughout the nuclei. Many granules occur clumped in small groups, thus leaving several unstained spaces in the nuclei (fig. 8, Pl. 37). A network does not seem to be present, but a few granules gave the appearance of being connected by threads. In no case were granules identifiable as chromatin observed outside the nuclear membrane nor were accessory nuclei shown in the cytoplasm.

In material stained with Mann's methyl-blue eosin the nuclei

of the early nurse-cells of *Thrinax mixta* were revealed as faintly basophil bodies in which small vacuoles may be present (fig. 3, Pl. 37). In a slightly older stage the vacuoles are greater in size and number, while the nucleolus is more strongly basophil (fig. 4, Pl. 37). Later, the nucleolus breaks up, liberating several basophil bodies containing vacuoles (fig. 5, Pl. 37). These in turn appear to fragment as, in the older nurse-cells towards the posterior end of the ovarioles, the nuclei contain several basophil bodies (fig. 6, Pl. 37). In *Allantus pallipes* the behaviour of the nucleoli of the nurse-cells is closely similar, and although the basophil bodies may remain in contact with the original nucleolus for some time, ultimately fragmentation takes place. The nucleolus and bodies originating from it are not so strongly basophil as in *Thrinax mixta*.

From the above account it will be seen that the nucleolus and basophil bodies correspond to the 'nuclear material', the behaviour of which was described in a former contribution (9); however, owing to the methods then employed, the chromatin granules could not be differentiated from the basophil material.

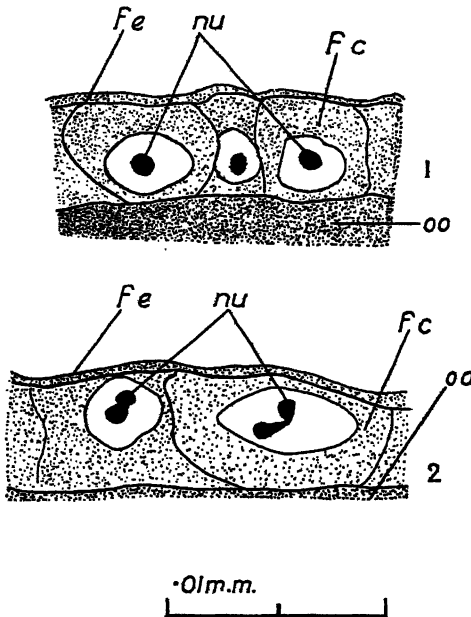
2. Follicle-cells.

The behaviour of the chromatin of the follicle-cells appears to closely resemble that of the nurse-cells already described. In the follicle-cells surrounding the early oocytes the majority of the chromatin granules are situated in the vicinity of the nuclear membrane. The structure of the nuclei, however, is more easily determined in the older cells.

Thus in *Allantus pallipes* the granules were seen to be distributed in a nuclear network in a like manner to those of the nurse-cells (fig. 11, Pl. 37), while in *Thrinax mixta* they occur scattered through the nucleus, some few appearing to be connected by a thread (fig. 10, Pl. 37). In the late oocytes after yolk-formation the follicle-cells become somewhat flattened and at the same time the nuclei lose their rotundity and become elongate (figs. 10 and 12, Pl. 37). In this stage the nuclear network of the *Allantus pallipes* follicle-cell nuclei is not present, the chromatin granules occurring scattered through the

nucleus as in *Thrinax mixta* (fig. 12, Pl. 37). The nucleoli of the follicle-cells of the two species of saw-flies examined, as revealed by material stained in Mann's methyl-blue eosin, are basophil (Text-fig. 1). In many of the cells surrounding the late oocytes the nucleoli have become broken up and are repre-

TEXT-FIGS. 1 AND 2.



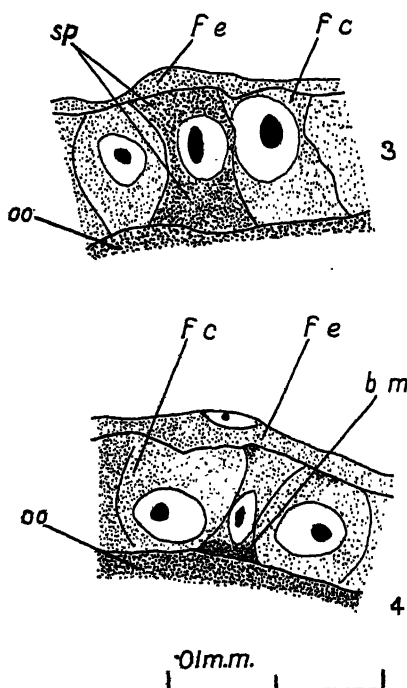
Thrinax mixta. Follicle-cells from young oocyte. *f.e.*, follicular epithelium; *f.c.*, follicle-cells; *nu.*, nucleolus; *oo.*, ooplasm.

Thrinax mixta. Follicle-cells from late oocyte. Lettering as in Text-fig. 1.

sented by large basophil masses; in most cases the latter remain in contact (Text-fig. 2).

In *Thrinax mixta* the cytoplasm of the follicle-cells is basophil. Many cells, however, proved to be more strongly basophil than their neighbours, while others contained a large amount of deeply basophil material situated between the cell nuclei and that part of the cell-membrane adjoining the oocyte

(Text-figs. 3 and 4). This material, no doubt, corresponds to the darkly stained granular material described in a former contribution (9), but in no case were granules observed passing from follicle-cell to oocyte. In *Allantus pallipes* the follicle-cells do not appear to contain this deeply basophil material.



Text-figure 3: *Thrinax mixta*. Showing follicle-cell more deeply basophil than its neighbours. *sp.*, unstained spaces in cytoplasm; other lettering as in Text-fig. 1.

Text-figure 4: *Thrinax mixta*. Showing deeply basophil material in follicle-cell. *bm.*, basophil material; other lettering as in Text-fig. 1.

Many of the follicle-cells contained unstained spaces which gave to the cytoplasm a vacuolated appearance, this condition being particularly noticeable in the deeply basophil material of some of the cells mentioned above (Text-fig. 3). Its significance was not determined.

3. Oocytes.

In *Allantus pallipes* the young oocytes at the proximal end of the ovarioles contained chromatin granules distributed on a nuclear network (fig. 9, Pl. 37), but in the older cells no chromatin was demonstrated by Feulgen's method, the nuclei containing only material which stained with light green. As previously stated the ovarioles of *Thrinax mixta* were more highly developed, consequently the oocyte nuclei of this material did not reveal the presence of chromatin. In neither species did the oxyphil or basophil nucleoli give the reaction for chromatin. If chromatin is passed out of the nuclei with the nucleolar extrusions of either species, then, owing to the large size of the basophil extrusions of *Thrinax mixta* and of the extruded basophil bodies of *Allantus pallipes*, its presence should be readily detected; an examination, however, failed to reveal the presence of chromatin in the ooplasm.

V. DISCUSSION.

In the material treated by Feulgen's method chromatin granules were not shown round the nurse-cell nuclei, nor could the presence of granules be detected in the cytoplasm; consequently, extrusion of chromatin from nurse-cell nuclei cannot take place in *Thrinax mixta* or *Allantus pallipes*. This shows clearly the danger of drawing conclusions concerning chromatin extrusions from material which is fixed and stained by the old methods. The whole question of such extrusions needs reinvestigation with modern technique such as Feulgen places in our hands. The nature of the perinuclear granules previously revealed in nurse-cell material stained in acid fuchsin and iron haematoxylin was not determined.

The large masses of 'nuclear material' in the nurse-cell nuclei previously demonstrated (9) are shown to consist of material originating from the basophil nucleolus, the behaviour of which was correctly described in a former contribution (op. cit.), although, owing to the methods then used, the exact nature of these bodies was not determined. The nucleolar bodies are not

extruded to the cytoplasm, but are apparently utilized for the nourishment of the developing eggs after the engulfment of the nurse-cell nuclei. During the process of fragmentation the nucleolar material appears to increase in amount as is evidenced by the large number of basophil bodies present in the nuclei of the fully formed nurse-cells.

The deeply basophil material present in many of the follicle-cells of *Thrinax mixta* confirms some previous observations on this phenomenon (op. cit.), but in the present investigations granules passing from follicle-cell to oocyte were not detected. It is possible that some of the larger granules previously described were in reality small albuminous yolk-globules which were subsequently shown to originate at the periphery of the oocyte (Gresson, 5). This confusion could not arise in material treated by the methods employed in the present study. Owing to the fact that the basophil material is situated in that part of the follicle-cells adjoining the oocyte, it may be that some substance is passed in solution into the ooplasm; however, no proof of this supposition was forthcoming.

The absence of accessory nuclei from the cytoplasm of the nurse-cells and from the ooplasm shows that former conclusions concerning the origin of these bodies from the nurse-cells, follicle-cells, and oocytes of the two species of saw-flies dealt with in this paper have to be withdrawn in the light of Feulgen's method. The writer, however, does not deny that accessory nuclei may exist in other species of saw-flies, as it has been shown that variation in their manner of origin occurs in related forms (Buchner, 1), and, more recently, that variations exist in the behaviour of the nucleoli of different species (Gresson, 4 and 5); hence it is not improbable that accessory nuclei, while absent in some, are present in other members of the group. It is evident that with certain technique the basophil and oxyphil nucleolar emissions bear a resemblance to accessory nuclei. The present contribution, however, shows clearly that in the species under investigation they do not contain chromatin but consist solely of nucleolar material. It is interesting to note that so long ago as 1920, and without the more recent technique, Hogben (6),

while discussing the nucleolar extrusions of the dragon-fly, *Libellula*, argues that the Hymenopteran accessory nuclei are nucleolar rather than chromatinic in origin; thus, 'the oogenesis of *Libellula* shows that bodies may be formed within the plasmosome itself which have as genuine a resemblance to true nuclei as the secondary "nuclei" of the Hymenopteran egg: this reinforces the evidence for regarding the latter as a product of the plasmosome; and the undoubted emission of nuclear material during oogenesis no longer necessitates the view that the integral continuity of the chromatinic organization of the nucleus is interrupted in the diffuse stage'.

The presence of a small amount of chromatin in the early oocytes of *Allantus pallipes* agrees with Ludford's findings for the oocytes of the mouse and *Limnaea stagnalis* (8); its subsequent disappearance in the older oocytes more closely resembles the condition described by Koch for Chilopods (7), where chromatin, although present in the oogonia, was not observed in the oocytes. Koch concludes that during oogenesis the chromatin undergoes a chemical change, while Ludford, in order to account for the small amount of chromatin present and for a faint purple colouration shown in some of the oocyte nuclei of *Limnaea stagnalis*, believes that the chromatin may be so finely dispersed as to render its detection by Feulgen's method impossible; on the other hand, these appearances may be due, he states, to a chemical change such as suggested by Koch. It would seem that the disappearance of the chromatin from the older oocytes of *Allantus pallipes* and its complete absence from the more highly developed ovarioles of *Thrinax mixta* would support Koch's view.

The absence of chromatin from the nucleoli and nucleolar extrusions agrees with Ludford's findings for the mouse and for *Limnaea stagnalis*, where chromatin could not be detected in the nucleoli.¹

¹ The writer's attention has recently been drawn to an abstract ('Jap. Journ. Zool.', vol. 2, no. 3, 1929) of a paper by K. Sato on the nucleolus of the bivalve, *Cristaria plicata*. Amongst other points Sato claims that the nucleolus is 'a reservoir of chromatin. In some cases (in degenerating

VI. SUMMARY.

1. By the use of Feulgen's 'nuclealreaktion' certain points of *Tenthredinid* oogenesis have been subjected to closer study. The chromatin of the early nurse-cells of *Allantus pallipes* exists in the form of granules, the majority of which occur close to the nuclear membrane. In the older cells a nuclear network appears in which is distributed granules of chromatin. In *Thrinax mixta*, where the ovarioles were more highly developed, the chromatin of the nurse-cells occurs as granules scattered through the nucleus; a nuclear network is not present, but certain granules appear to be connected by a thread. The granules which were shown to surround the nurse-cell nuclei (in material treated by Bensley's method and also by fixation in Bouin's picro-formol and subsequently stained in iron haematoxylin) and which were formerly regarded as chromatin emissions from the nurse-cell nuclei (9) were not revealed by Feulgen's technique. They therefore cannot be regarded as chromatin. Their precise nature and origin remains undetermined.

2. The nucleoli of the early nurse-cells of both species, as revealed by Mann's methyl-blue eosin, are faintly basophil. Later they break up into a number of basophil bodies which undergo fragmentation; formerly (technique and reference as in 1) the basophil nucleolus and the basophil bodies originating from it were termed 'nuclear material' undergoing fragmentation. While this basophil nucleolar material presents a fragmented appearance, it increases in amount as evidenced by the large number of basophil bodies present in the older nurse-cell nuclei. This material is utilized for the nourishment of the egg cells, for instance) it is formed from chromatin. The plasmosome is the denser part, from which the karyosome and afterwards loose chromatin is derived and vice versa'. The accessory nuclei are generally derived from the karyosome, sometimes from the plasmosome, but rarely from chromatin. Sato suggests that instead of the terms acidophil and basophil 'the non-committal terms deeply staining and lightly staining' be used. The writer has so far been unable to obtain an original copy of Sato's paper and consequently, owing to lack of detail regarding methods, &c., is unable to express a definite opinion on Sato's claims, but would suggest that these would probably require revision if the Feulgen technique were employed.

after the latter engulfs the nurse-cell nuclei. Nucleolar extrusions to the cytoplasm do not occur.

3. The behaviour of the chromatin of the follicle-cell nuclei is similar to that of the nurse-cell nuclei except that in *Allantus pallipes* the nuclear chromatin network as demonstrated by Feulgen's technique disappears in the older cells.

4. The nucleoli of the follicle-cells are basophil. They become broken up in the older cells, but in most cases the resulting masses remain in contact. Nucleolar extrusions to the cytoplasm do not occur.

5. The occurrence of deeply basophil material in the cytoplasm of the follicle-cells of *Thrinax mixta* stained with Mann's methyl-blue eosin, formerly described for Bouin fixed material stained in iron haematoxylin (9), suggests that some substance in solution may be passed into the ooplasm; extrusion of granules from the follicle-cells to the ooplasm does not take place.

6. The absence or non-visibility of chromatin (Feulgen's technique) from the oocytes of *Thrinax mixta*, and its disappearance from the older oocytes of *Allantus pallipes*, would indicate that the chromatin undergoes a chemical change during oogenesis such as suggested by Koch for *Chilopods*.

7. The oxyphil and basophil nucleoli of the oocytes do not react to Feulgen's technique for chromatin; this agrees with Ludford's findings for the mouse and for *Limnaea stagnalis*.

VII. CONCLUSIONS.

1. Feulgen's 'nuclealreaktion' shows clearly that visible chromatin is not extruded from the nuclei of nurse-cells, follicle-cells, or oocytes; consequently chromatin plays no part in the nourishment of the egg, except when it is engulfed with the nurse-cell nuclei; nor do accessory nuclei occur in the saw-flies under consideration.

2. A former conclusion (9) that chromatin or accessory nuclei were extruded from nurse-cell, follicle-cell, and oocyte has therefore to be withdrawn, but material stained with Mann's methyl-blue eosin confirms previous statements regarding the

darkly stained cytoplasm in certain follicle-cells, though it fails to show the extrusion of granular material from follicle-cell to oocyte.

3. The darkly stained masses in the nurse-cell nuclei formerly termed 'nuclear material' (9) now prove to be of nucleolar origin.

VIII. ACKNOWLEDGEMENTS.

I wish to express my thanks to Professor A. D. Peacock, in whose Department this work was carried out, for material, research facilities, and advice during the course of my investigations.

IX. REFERENCES.

1. Buchner, P.—"Die akzessorischen des Hymenoptereneises", 'Archiv f. Mikr. Anat.', Bd. 91, 1918.
2. Enslin.—"Die Tenthredinoidea Mitteleuropas", 'Deutsche Entom. Zeitschr.', Heft 7, 1912.
3. Feulgen, R.—"Die Neuclealfärbung", 'Abderhalden's Handbuch der biologischen Arbeitsmethoden', Abt. V, Lief. 213, 1924.
4. Gresson, R. A. R.—"Nucleolar Phenomena during Oogenesis in certain Tenthredinidae", 'Quart. Journ. Micr. Sci.', vol. 73, 1929.
5. ——"Yolk-formation in certain Tenthredinidae", *ibid.*
6. Hogben, L.—"Studies on Synapsis. III. The Nuclear Organization of the Germ Cells in *Libellula depressa*", 'Proc. Roy. Soc. "B"', vol. 92, 1921.
7. Koch, A.—"Morphologie des Eiwachstums der Chilopoden", 'Zeit. f. Zellforsch. u. Mikr. Anat.', Bd. 2, 1925.
8. Ludford, R. J.—"Studies in the Microchemistry of the Cell. I. The Chromatin Content of Normal and Malignant Cells, as demonstrated by Feulgen's Nuclealreaktion", 'Proc. Roy. Soc. "B"', vol. 102, 1928.
9. Peacock, A. D., and Gresson, R. A. R.—"The Roles of the Nurse-cells, Oocytes, and Follicle-cells in Tenthredinid Oogenesis", 'Quart. Journ. Micr. Sci.', vol. 71, 1928.

EXPLANATION OF PLATE 37.

The drawings were made by means of a Zeiss camera lucida and a Watson 'Service' Microscope. For all figs. a Reichert $\frac{1}{10}$ objective was used. The eye-piece was a Hawksley No. 4 \times 10.

LETTERING.

b.b., basophil bodies originating [from nucleolus; *c.*, cytoplasm; *c.gr.*, chromatin granules; *f.c.*, follicle-cell; *f.e.*, follicular epithelium; *n.n.*, nuclear network; *nu.*, nucleolus; *oo.*, ooplasm.

PLATE 37.

Figs. 3, 4, 5, 6, 8, and 10 from *Thrinax mixta*, all others from *Allantus pallipes*.

Fig. 1.—Early nurse-cells at anterior end of ovariole.

Fig. 2.—Nurse-cell from first nutritive chamber showing chromatin granules and network.

Fig. 3.—Nucleus of nurse-cell at anterior end of ovariole showing basophil nucleolus.

Fig. 4.—Later stage showing vacuoles in nucleolus.

Fig. 5.—Later stage showing basophil bodies which have originated from the nucleolus.

Fig. 6.—Nucleus of nurse-cell from fully formed nutritive chamber showing numerous basophil bodies. Note increase in size of nucleus.

Fig. 7.—Nucleus of nurse-cell from fully formed nutritive chamber showing chromatin granules and nuclear network.

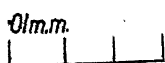
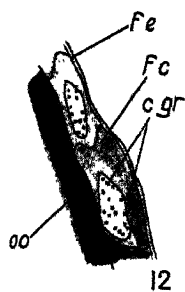
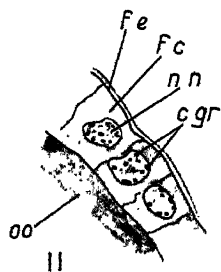
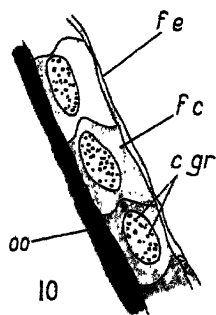
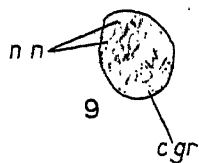
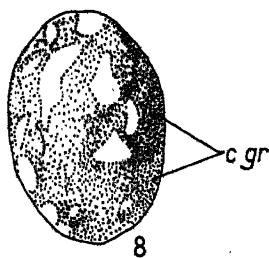
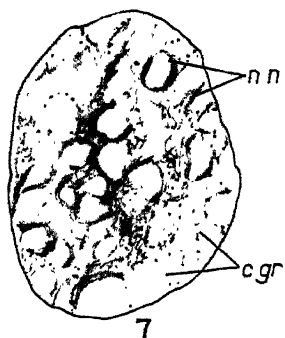
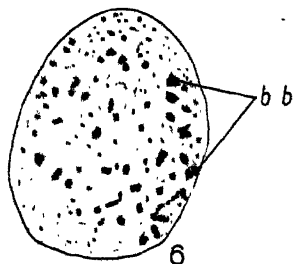
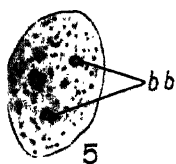
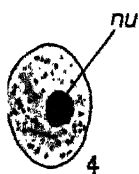
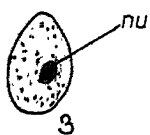
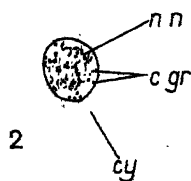
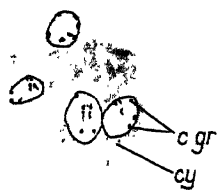
Fig. 8.—From late nurse-cell showing chromatin granules in nucleus.

Fig. 9.—Nucleus of early oocyte showing chromatin granules and nuclear network.

Fig. 10.—Showing chromatin granules in follicle-cell nuclei.

Fig. 11.—Follicle-cells; chromatin granules and network in nucleus.

Fig. 12.—Follicle-cells from late oocyte; chromatin granules in nucleus.



The Cytological changes observable in irradiated Bean Root Tips.

By

Ruth E. P. Patten, B.A., M.Sc. (Dubl.),

John Edward Cooney Scholar, Lady Margaret Hall, Oxford,

and

Sylvia B. Wigoder, M.A., M.D. (Dubl.),

Assistant Radiologist, Manchester and District Radium Institute.
(From the Department of Zoology, Trinity College, Dublin, and Rotunda Hospital, Dublin.)

With Plates 38-40.

INTRODUCTION.

IN our previous papers (21) we have fully described the gross structural alterations which X-rays produce in bean seedlings; we only briefly discussed the cytological changes, and it is our object in this paper to discuss these in greater detail, and the modifications produced by altering the dose.

PREVIOUS WORK.

The effect on cells of irradiation (both X-rays and radium) has been studied by various investigators. In 1904 Bergonié and Tribondeau (2), working with the testis of white rats, formulated their well-known law that 'Immature cells and cells in a state of active division are more sensitive to X-rays than are cells which have already acquired their fixed adult morphological or physiological characters'. In 1911 Paula Hertwig (8) described certain abnormalities of mitosis in irradiated *Ascaris* eggs. In 1927 Strangeways and his co-workers (17, 18, 19) found in tissue cultures that the period immediately preceding visible prophase is the most radio-sensitive, and Pekarek (14) in 1927 published a long paper dealing with the changes

produced in bean-tips as a result of X-radiation. During the last year Gatenby and his pupils (6, 7) described changes in the Golgi bodies of the testicular cells of irradiated guinea-pigs and insects.

MATERIALS AND TECHNIQUE.

The majority of experiments herein described were carried out on the same commercial brand of *Vicia faba* seeds, but towards the end, the stock was exhausted and so a different type of broad bean was used.

As in our previous work the beans were steeped in water at room temperature for 24 hours, then grown in damp sand for 3 days until the roots measured about 1.5 cm., after which they were irradiated. The conditions of X-radiation were: Phillip's metalix tube, Type E, 110 K.V. ($7\frac{3}{4}$ inch spark gap between point and plate) 4 M.A. and a distance of 23 cm. between the flat glass dish containing the beans and the anti-cathode of the tube. The beans were always covered with black paper during irradiation.

We divided our experiments into three groups.

First Series.—Irradiated for 8 minutes (in this paper we refer to this dose as heavy irradiation).

Second Series.—Irradiated for 4 minutes (in this paper we refer to this dose as medium irradiation).

Third Series.—Irradiated for 1 minute (in this paper we refer to this dose as light irradiation).

The tips from one or two beans from each of these series were cut 1, 5, 10, 15, 20, 30, 45 minutes, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3, 4, 6, 9, 12, 15, 18, 24, 36–40 hours, 2, 3, 5, and 7 days after irradiation, and sometimes at later periods. As a general rule changes found in any one root-tip were verified by cutting another root-tip at an exactly similar length of time after irradiation, and usually control beans were cut at the same time as the irradiated ones.

Either Bouin's fluid or corrosive acetic were used as fixatives. About 4 mm. of the tip of the root was left in the former for 12–24 hours, then dehydrated by leaving for $\frac{1}{2}$ –1 hour in each of the different grades of alcohol and then embedded in paraffin

in the usual way. The corrosive acetic was made by adding 2 cc. of glacial acetic acid to a 6 per cent. solution of corrosive sublimate, and the 4 mm. of root-tip were left in this for 24 hours; then washed in running water overnight, dehydrated and embedded as before. All the sections were cut thick. Two methods of staining were employed. The slides were brought down to water, and then placed on an electric plate which had been heated to 65° C. and 4 per cent. iron alum was poured over them from a pipette. They were left there for about 4 minutes, care being taken that they did not dry, more cold iron alum being pipetted over them as required. At the end of 4 minutes, when they were just beginning to steam, they were washed in cold water for a few seconds, replaced on the plate and cold 1 per cent. haematoxylin poured over them. They began to steam in 3 minutes, and the heater was then turned off. They were left for 8-10 minutes in the haematoxylin, more cold stain being added so that they were always well covered. They were washed in cold tap-water and differentiated in cold iron alum (4 per cent.) in the usual way.

The other staining method used was that of Feulgen's 'Neuklealreaktion' (5). The slides were brought to water, left in a beaker of HCL (heated to 60 C.) for 4 minutes, then left in fuchsin sulphurous acid for 3 hours, washed in water containing an excess of SO₂, counter-stained in a 2 per cent. alcoholic solution of light green for $\frac{1}{2}$ second, and then like the iron haematoxylin stained slides mounted in balsam. The Neuklealreaktion counter-stained in light green gives an intensely purple nucleus and green cytoplasm.

Very often the control and irradiated bean-tips were fixed in the same dish to ensure exactly similar conditions of fixation. When this was done the non-irradiated tip was cut a little longer than the irradiated one, in order to distinguish them.

EXTRACT FROM PROTOCOL.

First Series.

1. 5, 10, 15 minutes after irradiation.—No departure from normal.

20 minutes after irradiation.—Decrease in the number of dividing cells, most marked in anaphase and telophase. Some clumped metaphases.

30 minutes after irradiation.—Further reduction in number of dividing cells.

45 and 60 minutes after irradiation.—Still further decrease in number of dividing cells. Majority of metaphases clumped. Some abnormal anaphases.

90 minutes after irradiation.—Further reduction in number of dividing cells. Almost all metaphases clumped, some pseudo-amitotic figures, and 'gummy' mitoses. Telophase with abnormal distribution of chromatin substance.

2 and $2\frac{1}{2}$ hours after irradiation.—All metaphases clumped. Prophases still normal.

3 hours after irradiation.—More clumped metaphases than any other stage of cell-division.

6–12 hours after irradiation.—Prophases becoming progressively rarer. Diminution in number of metaphases (all clumped). Anaphase and telophase stages very rare and then abnormal.

15 hours after irradiation.—Only a few clumped metaphases present.

1 and 3 days after irradiation.—No mitosis present.

5–8 days after irradiation.—Some dividing cells. All stages present, mostly abnormal. Early disappearance of nuclear wall in prophase, and irregular arrangement of chromosomes. 'Gummy' mitosis and pseudo-amitosis. Many binucleate and multi-nucleate cells.

11 days after irradiation.—Decrease in number of dividing cells. Abnormalities as before.

12–19 days after irradiation.—Radicle had ceased to grow. No cell-divisions. Some multi-nucleate cells.

Second Series.

1, 5, 10, 15, 20, 30 minutes after irradiation.—No departure from normal.

45, 60 minutes after irradiation.—Slight decrease in number of dividing cells, some clumped metaphases.

90–120 minutes after irradiation.—Number of dividing cells definitely decreased, most marked in anaphase and telophase, the latter often abnormal. All metaphases clumped.

2½–15 hours after irradiation.—Progressive decrease in number of dividing cells, those present mostly prophase and clumped metaphase.

24 hours after irradiation.—Practically no dividing cells present.

2–3 days after irradiation.—Many cell-divisions present, with the exception of early prophase, mostly abnormal. Late telophase rare. Multi-nucleate cells abundant.

5–7 days after irradiation.—More multi-nucleate cells, but progressive diminution in number of cell-divisions.

8–13 days after irradiation.—No cell-divisions. Many multi-nucleate cells.

Third Series.

1–45 minutes after irradiation.—No departure from normal.

60 minutes after irradiation.—Slight decrease in number of dividing cells. A few chromosome bridges and dwarf nuclei, usually attached to the main nucleus.

90–150 minutes after irradiation.—Progressive decrease in number of dividing cells. All stages still found.

3–12 hours after irradiation.—Many chromosomes now show beaded appearance.

15–24 hours after irradiation.—Cell-division almost ceased, but all stages still seen in some sections. Later stages very rare.

36–48 hours after irradiation.—A few dividing cells.

All stages, mostly normal. Many bi- and multi-nucleate cells.

3-4 days after irradiation.—Many multi-nucleate cells, chromosome bridges and 'gummy' mitosis. A few normal cell-divisions.

16 days after irradiation.—Very many dividing cells, both normal and abnormal.

The most noticeable result of these experiments is the gradual decrease in the number of dividing cells. This phenomenon is most marked in the roots of beans irradiated for 8 minutes. Here a distinct decrease occurred 20 minutes after irradiation, and 15 hours later, mitosis had practically ceased, and this condition continued for the next four days. Generally 5-8 days after irradiation cell-divisions reappear, but disappear again as the seedling dies from 9-18 days after irradiation.

In the second series a slight decrease in dividing cells was to be observed 45 minutes after irradiation. Complete cessation never occurs, but almost complete cessation occurs in radicles fixed 24 hours after irradiation. Mitosis recommences 2-3 days after irradiation, but ceases again 10-12 days later, although death of the radicle does not occur for some days.

In the third series the variation in the number of dividing cells is much more gradual. Reduction in number of mitoses commences about 1 hour after irradiation, but the numbers never fall as low as in the other series, the minimum being reached about 24 hours later. Recovery begins 30-36 hours after irradiation and numerous cell-divisions are found in material fixed 16 days after irradiation.

To ascertain the cause of the disappearance of mitosis, it is at first essential to note how X-rays might affect the cell and its nuclear organization.

Cells 'hit' by X-rays in some phase of division might be acted on in one of the following ways. They might :

(1) Continue to divide at the normal rate with or without nuclear abnormalities.

(2) Continue to divide, but at a slower rate, with or without nuclear abnormalities.

(3) Be inhibited from completing mitosis.

Similarly those cells which are in the interphase¹ at the time of irradiation, may :

(4) All undergo mitosis either normally or abnormally.

(5) Some undergo mitosis either normally or abnormally.

(6) None undergo mitosis.

That cells in the dividing state continue the mitotic process in a normal manner is an untenable hypothesis, since abnormalities are found in the metaphase 20 minutes after irradiation, and these cells could hardly have been in the resting state at irradiation, unless the whole process of division had been speeded up, and we can find no evidence in our own work, or in that of others, to support this view ; indeed the whole bulk of evidence is more in favour of the theory that X-rays produce abnormalities in the dividing cell and may retard or inhibit (18, 19) the whole process.

Our investigations lend some support to the conception of a slowing down of this process. This is given by the relatively large number of clumped metaphases seen in the first and second series from 3-15 hours after irradiation. In beans irradiated for 8 minutes, 9-15 hours after irradiation, this phase is practically the only one seen, and the only inference that can be made is that the cells are delayed for a long period in this state. According to Sharp (16) a certain degree of clumping of the chromosomes normally occurs at metaphase in the mitosis of many animals and plants, but is of such a short duration that it is frequently overlooked in studies on mitosis. The ultimate fate of these clumped metaphases will be discussed later.

There is no evidence in support of the third suggestion, that the dividing cells are inhibited from completing the cycle ; for in the first minute series, at least, cut 1-3 days after irradiation, no phases of cell-division can be seen. These cells must, therefore, have finished their division unless the nucleus disintegrated

¹ By 'interphase' we mean the period between the completion of one division and the beginning of the next, whether it be of long or short duration.

or passed straight into the resting state. There is no vestige of evidence for either proposition.

It is obvious that the fourth suggestion is untrue since mitosis definitely decreases in number shortly after irradiation, and this could not occur if the normal number of cells entered a fresh division cycle.

From a careful comparison of series 1, 2, and 3, taking into account the rate at which mitotic figures disappear, it seems that few, if any, cells pass into mitosis in the first series, some in the second, and a considerable quantity in the third series.

The most plausible explanation for the gradual reduction in the number of cell-divisions seems to us to be that cells in division at the time of irradiation complete the process, probably at a slower rate than is normal, but the form their mitosis takes may be of an unusual kind. Cells in interphase, with the larger doses of X-rays, are prevented for at least a considerable time from dividing, while smaller doses damage them to a less extent.

We admit that this explanation does not tally with the general belief that the dividing cell is more affected by X-rays than the resting or interphase cell. It must, however, be taken into consideration that the cells of the meristematic region of a root-tip are passing into division at such a rapid rate, that the interphase can hardly be spoken of as a resting stage, being more correctly a relatively short period between telophasic reconstruction of the nucleus and the beginning of prophase. Nevertheless, it certainly is peculiar that the interphase should be the critical stage, and we can only suggest that during this period some physiological factor which provides the impetus for division is disturbed, and is apparently restored before the nuclei recover their power of division.

Strangeways and Oakley (19) cite the stage which immediately precedes visible prophase as being the most radiosensitive. Possibly this would be the time at which the lack of this 'physiological impetus' is felt. Moreover, if the greater portion of the irradiation period occurred, either before or after the cell had entered this critical period, the effect of X-rays would be less obvious, and instead of the cell being completely inhibited

from dividing, it would do so to a limited extent, the amount of division possible for it to undergo depending on how much of the irradiation period coincided with the sensitive time.

DISCUSSION ON ABNORMALITIES OBSERVED.

In the first and second series, the first and most striking nuclear abnormality is the appearance of what we have called 'clumped metaphases' (fig. 1, Pl. 38). In these the chromatinic material is seen to be gathered into a dense mass in which discrete chrosomes can seldom be observed, though the degree of condensation varies. The spindle fibres can generally be seen, but if they are not present it is impossible to tell whether these structures are prophases or metaphases.

In normal bean-tips, similar stages are occasionally met with. As mentioned above, this aggregation of chromosomes in late prophase or early metaphase probably normally occurs in the mitosis of *Vicia faba*, but is of extremely short duration.

It is apparent, from the relatively large number of these clumped metaphases, and from the fact that material cut at 12-15 hours after heavy doses, showed practically no stages of division except these clumped metaphases, that there must be some very considerable delay in this phase—6-12 hours after irradiation prophases become progressively rarer, thus there may be a delay in the metaphase for at least 3 hours. It seems, from the fact that these abnormalities appear 20 minutes after irradiation, that they arise from cells which received their initial injury in prophase or immediately before prophase. They succeeded in passing, more or less, normally into the early metaphase, but the mechanism of division being disturbed were held up at this stage. Similar condensations of the chromatinic substance have been observed by many workers after the application of various agents (for example, Nemec (12) after the application of chloral hydrate).

Strangeways and Oakley found clumped metaphases in tissue cultures, which had been allowed to incubate for 80 minutes after irradiation. They observed that nuclei in this stage

disintegrated and never completed the division. Disintegration of the clumped metaphase was never found in the bean root-tips. There is a distinct interval of days between the disappearance of these stages and the appearance of disintegrating cells. Further, since they are only found in material fixed less than 24 hours after irradiation, it seems that they finally pass out of this stage; occasionally nuclei in a state which can only be regarded as the passage of these metaphases into anaphase are found. As 'pseudo-amitotic' figures (figs. 2, 3, 4, Pl. 38) appear 30-40 minutes after these metaphases are first found, it would seem they are in some way connected with one another; it is highly probable that the clumped metaphases pass into these forms and so complete their division. These pseudo-amitotic figures have often been described and their nature discussed—Nemec (12), Sakamura (15), Pekarek (14). Our investigations are entirely in agreement with the findings of Pekarek (14), and there seems to be no doubt that these are not true amitotic figures; we consider them as a sequence of 'clumped metaphase', the aggregate mass of chromosomes, being drawn out into the dumb-bell shaped or more usually C-shaped pseudo-amitotic forms (fig. 3, Pl. 38). After light doses of X-rays, the first abnormalities which appear in large numbers are the 'chromosome bridges' (figs. 5, 6, Pl. 39). The chromosome bridges are seen in late anaphase and telophase. The majority of the chromosomes gives rise to the normal diaster appearance, but the poles remain connected by one or more elongated chromosomes which failed to be included with the rest of the chromatinic substance. These bridges may vary in size from fine threads of chromatin, which can hardly be distinguished from the spindle-fibres except in the slides stained by the Neuklealreaktion, when the green stained spindle-fibres can easily be distinguished from the purple chromatin. In the other cases the chromosome bridges may be very thick; while, again, up to 3 or 4 such bridges have been seen in one diaster stage. Sometimes these bridges are incomplete, as though gradually being absorbed into the polar masses. Occasionally the phragmoplast or cell-plate is found in which the chromosome bridge

is still apparent, and even in cells with telophasic reconstruction almost complete this abnormality may be seen.

These structures are not confined to irradiated material, and were occasionally seen in the control sections.

A peculiar observation is that after heavy doses, these chromosome bridges, which are then less numerous than after the light dose, do not appear for some days after irradiation, in fact, during the recovery period (2-3 days after a 4-minute dose, 5-8 after an 8-minute dose).

The term 'gummy mitosis' (figs. 6, 7, 8, Pl. 39) has been applied to those abnormal anaphases when one or more chromosomes remain at the equatorial region while the rest of the chromosomes are separated and pass in the usual way to the poles. This type of abnormality has often been described (Mottram (13), Pekarek (14), Strangeways and Oakley (19)).

The division of these abnormalities under the terms 'chromosome bridges' and 'gummy mitosis' is more or less arbitrary; they are both essentially an asymmetrical distribution of the chromatin, the 'gummy mitosis' being the abnormality in a more advanced degree. 'Gummy mitoses' are a characteristic feature during the period of partial recovery after the heavier doses, but are seldom present following light irradiation. Another abnormality which is found during the recovery period in all the series is the quite irregular distribution of the chromosomes (fig. 10, Pl. 40). It appears that the nuclear wall breaks down early in prophase, the spindle often fails to appear, and the chromosomes wander off in various directions; in other cases a spindle is formed, but the chromosomes fail to migrate up the fibres, and pass into the cytoplasm.

In the third series the chromosomes often showed a beaded appearance (fig. 9, Pl. 40) (Strangeways, Oakley, Pekarek). This abnormality occurs $1\frac{1}{2}$ -2 hours after irradiation; it seems as though the chromosomes were dividing into component parts or even disintegrating, but there is no evidence that the latter interpretation is correct, since no nuclear degeneration appears at this early period.

The appearance of bi- and multi-nucleate cells appears to be

a general sequence to irradiation (Strangeways (17, 18, 19), Gatenby and Wigoder (6, 29), Komuro (9), Canti (3), &c.). These are cells which contain two or more nuclei, contiguous or separated from one another, sometimes similar, but generally dissimilar in size. After the light dose of X-rays the dissimilarity in size is very great, one of the nuclei being almost normal in size, and the others so small that they might be termed dwarf nuclei.

After heavy irradiation, multi-nucleate cells appeared at the time of partial nuclear recovery, i.e. 5-8 days after irradiation for 8 minutes and 2-3 days after irradiation for 4 minutes, while a few dwarf nuclei are seen so soon as 6 hours after the light dose. Many theories have been propounded to account for their origin (Strangeways (17), Komuro (9), Sakamura (15), Kroenicke (10), Pekarek (14), &c.). In mammalian tissue, and tissue-cultures of chick embryo, coalescence of neighbouring cells which have undergone cytolysis is one possible explanation (Strangeways (17, 18, 19), Gatenby and Wigoder (6, 20)), but in plant material there is no evidence of cell-fusion.

From a study of our material several possible explanations suggest themselves. Amitosis and budding with the suppression of cell-wall formation have been suggested, but the dumb-bell shaped figures which have an amitotic appearance are, we think, the sequence to clumped metaphase. Further, the phragmoplasts are sometimes found in these cells (fig. 2, Pl. 38). There appears to be better evidence for the view that budding from the parent nucleus may occur. In the beans irradiated for 1 minute, one or more finger-like projections of the nucleus were frequently seen, and were most distinct in the sections stained by Feulgen's method. Sometimes a constriction was seen at the base of these projections, and as in the same material dwarf nuclei appeared in very close proximity to the parent nucleus, we thought that budding had occurred. However, on comparing control material which had been similarly fixed and stained, the same finger-like projections were present, but as no dwarf nuclei were seen, we came to the conclusion that the latter did not arise by budding, but were probably due to non-inclusion

of one or more chromosomes during the reformation of the daughter nuclei after division; these discrete chromosomes forming the dwarf nuclei. After the heavy doses, as previously mentioned, these supernumerary nuclei more nearly approach the normal size. As in this material the chromatinic substance which fails to pass to the poles in the numerous 'gummy mitoses' is of fairly large amount, it can be understood that the nuclei which will be derived from such an abnormal mass would be of moderate dimensions. Since, after light irradiation, the characteristic abnormality of division is the chromosome bridge, from which the chromatinic mass which fails to be included is small, and often adherent to the main mass at the poles, it can readily be seen that the resultant nucleus will be small and possibly in close proximity to the parent. In support of this hypothesis is the observation that the associated phenomena of 'gummy mitosis' and large secondary nuclei appear at about the same time (5-8 days after heavy irradiation, 2-3 days after medium doses), while the occurrence of later chromosome bridges and dwarf nuclei appears about 3-6 hours after light irradiation. Further possibilities for the formation of bi-nucleate cells are:—the suppression of cell-wall formation following either normal or asymmetrical division; while either bi- or multi-nucleate cells may be formed by the suppression of cell-wall formation following any abnormal division, but especially gummy mitosis or chromosome bridges; or by the reformation of two or more nuclei after irregular dispersion of the chromosomes.

Komuro (10) suggests the division of bi-nucleate cells might give rise to multi-nucleate cells, but as we have never seen a bi-nucleate cell in process of division we think that this is an extremely unlikely cause.

The changes produced by X-rays fall naturally into two groups. The first group occurs immediately after irradiation, and then, after a period of time depending on the size of the dose, the second group of changes (those of the recovery period) appear. As we have mentioned, after heavy irradiation there is a period of days during which no cells divide; this is followed by

partial recovery when some dividing cells are again seen ; while in those irradiated for 4 minutes the damage is not so great, and so recovery takes place sooner. In the beans X-rayed for 1 minute recovery occurs very rapidly.

The immediate effect after 8 minutes irradiation occurs in those cells which had commenced or were about to commence their division at the time of irradiation, and were able to complete it in a more or less normal manner, while in those irradiated for 1 minute the number of dividing cells is augmented by some of the interphase cells which are still able to pass into division. Clumped metaphase was the most constant abnormality after heavy irradiation, and chromosome bridges after light irradiation. These cells, together with those which were in the critical period at the time of irradiation, then went into a resting state, and did not again divide until the effect of irradiation had passed off, when they gave rise to the second group of abnormalities such as 'gummy mitosis' and multi-nucleate cells.

Alberti and Politzer (1) divided the sequence of events after irradiation into three stages. In the first, which continued for about 6 hours irrespective of the dose, degeneration of nuclei, and reduction in the number of dividing cells occurred. The second stage varied in duration with the size of the dose, and during it no cell-division took place. Finally, the third was one of partial recovery, and showed abnormalities of cell-division. Their classification agrees more nearly with our views than does the hypothesis of Pekarek (14), who believes that irradiation acts in one way on nuclei which are already in mitosis at the time of irradiation, and in completing their division give rise to the 'Primary effect' or his 'P Mitosen', and in another way on nuclei which at the time of X-radiation were in the condition of interphase, their subsequent division giving rise to the 'Secondary effect' or his 'S Mitosen'. In the material irradiated for 1 minute we find that the immediate effect must have been produced in cells which were both in the interphase and division stage at the time of irradiation ; and the 'recovering effect' must have been produced in similar cells or the product of similar cells. We consider that the difference between im-

mediate and recovery effect is more fundamental than is that of the 'P and S Mitosen' of Pekarek (12).

In the first and second series the immediate and recovery effects almost coincide with Pekarek's 'P and S Mitosen'; the immediate effect is confined to cells which were in division at the time of irradiation ('P Mitosen'), while the phenomena of the recovery period are largely the product of cells in the interphase at the time of irradiation ('S Mitosen'). Undoubtedly there is a certain degree of difference in the changes produced in the cells in the interphase and in active division at the time of irradiation, but the disparity in changes after irradiation, of cells in different phases of division would be just as great. The only apparent change possible in cells at early telophase or anaphase during irradiation is the suppression of cell-wall formation and at metaphase irregular distribution of the chromosomes ('gummy mitosis' and chromosome bridges), while if in prophase during irradiation, the whole mechanism of cell-division may be altered (i.e. no spindle formation), the chromosomes may fail to split longitudinally, or all the chromatin may form a dense mass. It is hard to see what relatively greater visible abnormality can be produced in cells which were either in interphase or immediately preceding visible prophase at the time of irradiation, though certainly after the heavy irradiation the physiological processes of the cell are disturbed in such a measure that division of the cell is not possible for a considerable time.

We have described and discussed the changes produced in irradiated bean-tip nuclei, but the main problem is still unsolved. In what way is the cell injured so that it is unable to function normally? Since it has been shown in work carried out in this laboratory on male lepidopterous germ-cells (7) that alterations are produced in the Golgi bodies 16 hours after irradiation of a nature to cause them to produce abortive acroblasts in the spermatocyte stage, and that 6 hours after irradiation the mitochondria tended to run together, although the nucleus showed no injury, it is obvious that some vital process of the cell is injured, and this in turn produces these abnormalities of nucleus

and cytoplasmic inclusions. Microscopic investigation using the most up-to-date cytological methods can reveal no more fundamental injury than has been described in these works, and the solution of the problem seems at present to be with the bio-chemist and the bio-physicist.

The entire expenses of these investigations were met by grants from the Medical Research Council, the Royal Irish Academy, and the Royal Society of London, whom we cordially thank. We are grateful to Dr. Solomons, the Master, and Dr. M'Donogh, the radiologist of the Rotunda Hospital, who gave us every facility for carrying out these investigations, and to Mr. D. B. Bradshaw for his help in the translation of numerous articles. Finally, we thank Dr. J. Brontë Gatenby for his interest, encouragement, and help.

SUMMARY.

1. The effect of X-rays is divisible into two periods, one showing the immediate effect, the second period being one of recovery.
2. The immediate effect includes gradual cessation of mitosis, and the production of abnormalities in those cells which were in the process of division or were about to divide at the time of irradiation.
3. The length of time during which no cells enter into mitosis depends on the size of the dose.
4. During the recovery period some cells again enter into mitosis but their division is usually of an abnormal type.
5. Irradiation acts on cells in the interphase by interfering with some physiological process. These cells are then unable to enter into mitosis until the effect of irradiation has passed off.

BIBLIOGRAPHY.

1. Alberti and Politzer, G.—“Über den Einfluss der Röntgenstrahlen auf die Zellteilung”, ‘Arch. f. mikroskop. u. Entwicklungsmech.’, 100, 83–109, 1923.
- 1a. ——— ‘Über den Einfluss der Röntgenstrahlen auf die Zellteilung. II. Mitt Ebenda’, 103, 284–307, 1924.
- 2a. ——— ‘Strahlentherapie’, 1923 and 1924; see also Levitt.
2. Bergonié and Tribondeau.—‘C. R. Soc. Biol.’, 5, 400 (1904), C. R. December 10, 1906 see Colwell and Russ.

3. Canti, G. R.—“Cinematograph demonstration of living tissue-cells growing in Vitro” (shown before the Leinster Branch of the B.M.A., May 1929), ‘Arch. f. exper. Zellf.’, **6**, 1928.
- 3a. Canti, G. R., and Spear, F. G.—“The Effect of Gamma Irradiation on Cell Division in Tissue Culture in vitro”, ‘Proc. Roy. Soc. B’, **102**, 1927.
4. Colwell and Russ.—‘Radium X-rays and the Living cell.’ Bell & Sons, 1924.
5. Feulgen, R.—“Die Nuclealfärbung.” Abderhalden’s ‘Handbuch der biologischen Arbeitsmethoden’, Abt. V, Lief. 213, pp. 1055–73, 1924.
6. Gatenby, J. B., and Wigoder, S. B.—“The Effect of X-radiation on the Spermatogenesis of Guinea-pigs”, ‘Proc. Roy. Soc. B’, **104**, 1929.
7. Gatenby, J. B., Mukerji, R. N., and Wigoder, S. B.—“The Effect of X-radiation on the Spermatogenesis of *Abraxas Grossulariata*”, ‘Proc. Roy. Soc. B’, **105**, 1929.
8. Hertwig, Paula.—“Durch Radiumstrahlen hervorgerufene Veränderungen in den Kernteilungsfiguren von *Ascaris megaloccephala*”, ‘Arch. f. mikros. Anat.’, **77**, 301, 1911.
9. Komuro, H.—“Preliminary note on the cells of *Vicia faba* modified by Röntgen Rays and their resemblance to tumour cells”, ‘Botan. Mag. Tokyo’, **36**, 41–5, 1922.
- 9a.—“The cells of *Vicia faba* modified by Röntgen rays and their resemblance to malignant cells, with the cytological observations of Tumours”, ‘Japanese Journ. Botany’, **2**, 133–56, 1924 B.
10. Koenicke, M.—“Über die Wirkung von Röntgen- und Radiumstrahlen auf pflanzliche Gewebe und Zellen”, ‘Ber. d. Dtsch. botan. Ges.’, **22**, 404–15, 1915.
11. Levitt, W. M.—“The beginnings and present position of deep X-ray therapy”, ‘St. Bartholomew’s Hospital Report’, **60**, 1927.
12. Nemec, B.—“Über die Einwirkung des Chloralhydrats auf die Kern- und Zellteilung”, ‘Jahrb. f. wiss. Botanik’, **39**, 1904.
13. Mottram, J. C.—“On the action of beta and gamma rays of radium on the cells in different states of nuclear division”, ‘Arch. of the Middlesex Hosp.’, **30**, 98–119, 1913.
14. Pekarek, J.—“Über den Einfluss der Röntgenstrahlen auf die Kern- und Zellteilung bei Wurzelspitzen von *Vicia faba*”, ‘Sonderabdruck aus Planta, Archiv für wissenschaftliche Botanik’, **4**, H. 3, S. 299–57, 1927.
15. Sakamura, T.—“Über die Einschnürung der Chromosomen bei *Vicia Faba*”, ‘Bot. Mag. Tokyo’, **29**, 287–300.
16. Sharp, L. W.—“Somatic Chromosomes in *Vicia Faba*”, ‘La Cellule’, **29**, 297–331.
17. Strangeways, T. S. P.—“Observations on changes seen in Living Cells during Growth”, ‘Proc. Roy. Soc. B.’, **94**, 1923.

18. Strangeways, T. S. P., and Hopwood, F. L.—“The effects of X-rays upon Mitotic cell-division in Tissue Culture in vitro”, ‘Proc. Roy. Soc. B.’, 100, 1926
19. Strangeways, T. S. P., and Oakley.—“The immediate changes observed in tissue cells after exposure to soft X-rays while growing in vitro”, *ibid.*, 95, 1923.
20. Wigoder, S. B.—“The effect of X-rays on the testis”, ‘Brit. Journ. of Radiol.’, 2, New Series, no. 17, May 1929.
21. Wigoder, S. B., and Patten, R. E. P.—“The effect of Röntgen Rays on Growing Seedlings”, Proc. Roy. Irish Academy, vol. xxxix, section B, no. 9, 1929.
- 21 a.—“Variations in the Growth of Irradiated Bean Roots,” ‘Brit. Journ. of Radiol.’, vol. ii, no. 24, 1929.

DESCRIPTION OF PLATES 38-40.

Taken with a Leitz Mikam Camera.

LETTERING.

B.C., Beaded chromosomes; *C.Br.*, Chromosome bridges; *C.M.*, Clumped metaphase; *G.M.*, ‘Gummy’ mitosis; *I.C.*, Irregular arrangement of chromosomes; *M.C.*, Multinucleate cell; *P.A.*, Pseudo-amitosis.

Fig. 1.—From bean-tip fixed 3 hours after irradiation for 8 minutes showing clumped metaphase.

Fig. 2.—From bean-tip fixed 3 hours after irradiation for 8 minutes showing pseudo-amitosis.

Fig. 3.—From bean-tip fixed 2 hours after irradiation for 8 minutes showing pseudo-amitosis.

Fig. 4.—From bean-tip fixed 15 hours after irradiation for 1 minute showing pseudo-amitosis with formation of cell-wall.

Fig. 5.—From bean-tip fixed $2\frac{1}{2}$ hours after irradiation for 1 minute showing chromosome bridge.

Fig. 6.—From bean-tip fixed 4 hours after irradiation for 1 minute showing chromosome bridge.

Fig. 7.—From bean-tip fixed 5 days after irradiation for 4 minutes showing gummy mitosis (*G.M.*¹ shows chromosomes already at the pole, *G.M.*² shows chromosomes remaining on equatorial plate).

Fig. 8.—From bean-tip fixed 8 days after irradiation for 8 minutes showing gummy mitosis with tri-polar arrangement of the chromosomes.

Fig. 9.—From bean-tip fixed 3 hours after irradiation for 8 minutes showing beaded chromosomes in abnormal anaphase.

Fig. 10.—From bean-tip fixed 5 days after irradiation for 4 minutes showing irregular arrangement of the chromosomes.

Fig. 11.—From bean-tip fixed 5 days after irradiation for 4 minutes showing multi-nucleate cells.

Fig. 12.—From bean-tip fixed 15 hours after irradiation for 1 minute showing multi-nucleate cells.



1

-CM

CM



2

PA



3

PA

PA



4

PA



5



6



GM¹

GM²

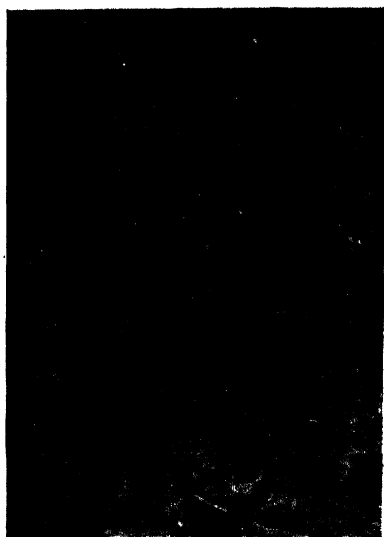
GM





9

BC



IC

10



MC

11



MC

MC

MC

12



On a new Hermaphrodite Syllid.

By

E. S. Goodrich

Linacre Professor of Zoology and Comparative Anatomy in the University of Oxford.

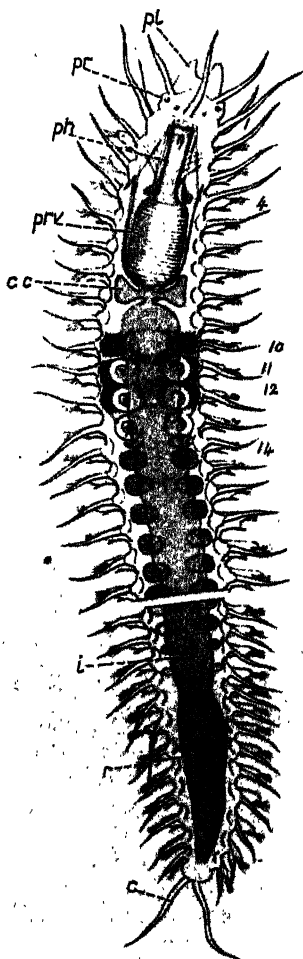
With 12 Text-figures.

THE worm described in this paper was found by me in material collected by the fishermen of the Zoological Station at Naples from the sandy bottom near the western shore of the bay of Naples. Although small it is fairly conspicuous, being milky white in colour and active in its movements. It belongs to the genus *Pionosyllis*, and like most of its near relatives bears its young attached in pairs on the segments of the middle region of its body, as admirably described and figured by Pierantoni (5). The breeding season begins in May, and the specimens I collected during my visit to Naples last April were not fully mature. To Mr. J. Z. Young, however, I am indebted for mature and embryo-bearing specimens collected in June.

Most of the structure described below was made out on the living worms, and these observations have been confirmed and completed with the help of whole preparations and sections. The best results were obtained with material preserved in Bouin's fixative. Sections were stained in carmine and picro-nigrosin, Ehrlich's haematoxylin, iron-haematoxylin, and Mann's methyl-blue eosin.

As shown in Text-fig. 1, this little Syllid has the characters of the genus *Pionosyllis* Malmgren (Pierantoni, 5; Fauvel, 2). The prostomial palps are fused only at their base; the long slender tentacles and cirri are not moniliform; there are three prostomial tentacles, two pairs of peristomial tentaculiform cirri, and a long dorsal and short ventral cirrus to each parapodium. The pharynx is armed with one large tooth, and its anterior

TEXT-FIG. 1.



Pionosyllis neapolitana, dorsal view of full-grown specimen, enlarged. Some segments have been cut out after the nineteenth. *c*, cirrus of pygidium; *cc*, caecum; *i*, intestine; *ph*, pharynx; *pl*, palps; *pr*, prostomium; *prv*, proventriculus; *r*, rectum.

edge is smooth. The compound chaetae are provided with an elongated distal segment (Text-fig. 8 A). The full-grown worm reaches a length of about 4 mm., with about thirty-eight

segments. But it differs strikingly from other recorded Pionosyllids in being hermaphrodite, and is remarkable for the complexity and constancy of structure of its reproductive organs.¹

The first nine segments (counting the peristomial segment as the first, and the first setigerous segment as the second) are sterile, but the tenth, eleventh, and twelfth segments are seen to be filled with spermatozoa in all full-grown individuals, and even in those which have not yet attained full size. From the thirteenth segment backwards, throughout the region of the true intestine, each segment contains a pair of ovaries. The posterior segments in the region of the rectum are again sterile.

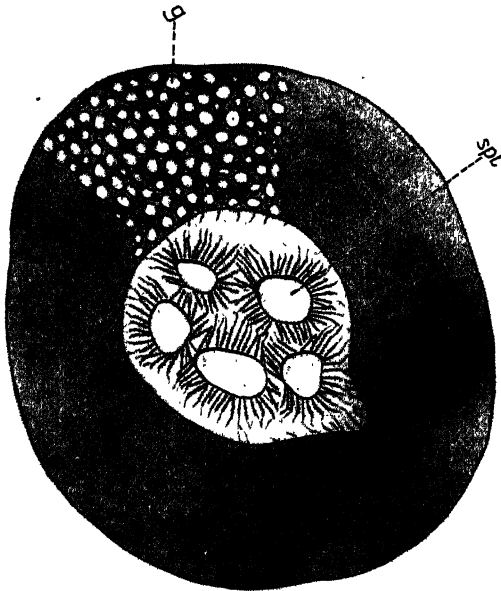
Segments 10, 11, and 12, which become filled with sperms, are closed by complete thin transverse septa preventing the spermatozoa from spreading to neighbouring segments. In adult specimens the coelomic cavities of these three segments become so packed with spermatozoa in all stages of development that they appear opaque and conspicuous under the microscope. The testes themselves, on the other hand, are obscured and difficult to see at this stage except in sections. A pair of testes is present in each of the segments 10, 11, and 12, and they appear as proliferations of the coelomic epithelium near the ventral edge of the longitudinal muscle-bands (Text-fig. 3). The ripe spermatozoa spread throughout the cavity of the segment and into the parapodia, but tend to gather at its outer posterior corners. Here they are accumulated by the action of the cilia of the funnels of the sperm-duets down which they are eventually driven.

The three pairs of sperm-duets are all alike in structure.

Hermaphroditism is not unknown among the Syllids. In addition to the case of *Syllis corruscans*, which is said to produce male and female buds, two species of the genus *Grubea* have recently been recorded as normal hermaphrodites. These are *Grubea protandrica*, incompletely described by Du Plessis (1) from the French Mediterranean coast, and *Grubea pusilloides*, described by Haswell (4) from Port Jackson. Of these the last species resembles most *Pionosyllis neapolitana*, since it has two male segments and several more posterior female segments. But in other respects it seems to differ widely from the worm here described.

They open respectively into the three successive male segments. Each sperm-duct has a funnel provided with actively moving cilia on the anterior face of the septum closing the male segment posteriorly. The funnel leads backwards through the septum

TEXT-FIG. 2.

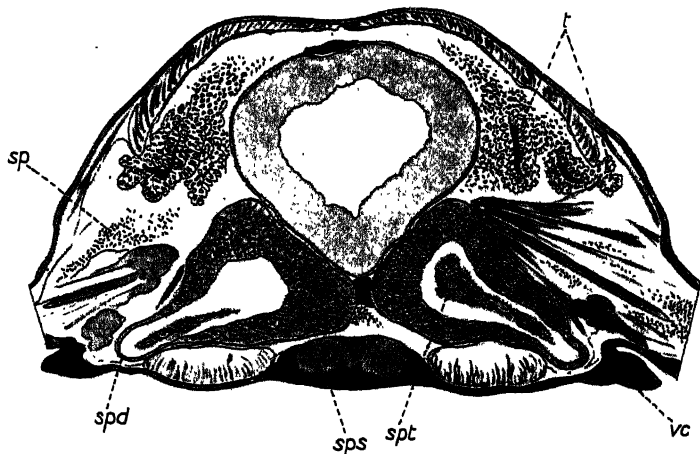


Much enlarged optical section drawn from life of a sperm-sac containing spermatophores, *spt*. In a portion of the wall are shown the refringent granules, *g*.

to a coiled tube, which suddenly expands into a nearly spherical sperm-sac, and narrows again to a thin walled ciliated tube (Text-fig. 5). The latter runs outwards to open near the base of the ventral cirrus. The sperm-sac has a thick wall composed of a single layer of large cells packed with large highly refringent granules (Text-figs. 2 and 3). So numerous are these granules that the six sacs are very refringent and conspicuous in the living worm. The inner surface of the sac is ciliated, at all events near the entrance and exit of the narrow duct. Lying in the cavity of the sac may be seen in the living a number of sperm-

tophores gyrating rapidly, being kept in motion by the cilia of the wall. More spermatophores generally are present in the duct to the exterior. The spermatophores in each sac vary in size and number—from one to six. Each spermatophore con-

TEXT-FIG. 3.



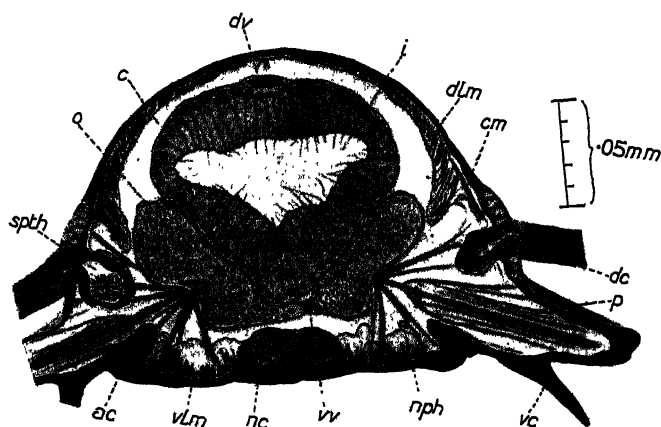
Transverse section of a male segment (12th). *sp*, ripe spermatozoa in coelom; *spd*, sperm-duct to exterior; *sps*, wall of sperm-sac; *spt*, mass of spermatophores and coagulum; *t*, testis partly on blood-vessel; *vc*, base of ventral cirrus. Magnification as in Text-fig. 4.

sists of a central refringent mass surrounded by spermatozoa fixed to it by their 'head' end and with 'tail' outwards. There can be little doubt that the central mass is made up of the same substance as the granules in the wall. They stain in the same way—green with picro-nigrosin, red with eosin; but do not take up nuclear stains such as carmine or haematoxylin. Sections of the wall of the sperm-sac show that the granules are distributed throughout the cytoplasm of the cells (Text-fig. 3). Each granule appears to be composed of a larger more darkly staining mass partially enclosing a paler and smaller sphere.

As already mentioned, from the thirteenth segment backwards, throughout the region of the true intestine, there is a

pair of ovaries in each segment (Text-fig. 1). The ovary is derived from the coelomic epithelium at the ventral edge of the longitudinal muscle-band and at a level half-way between the parapodium and the front septum. This can be seen clearly when the ovary is little developed (Text-fig. 6); but, as it grows, one central oocyte becomes very large, loaded with fine granules of yolk, and spreads downwards below the intestine (Text-figs. 1 and 4), so that the right and left ovaries of a segment tend to

TEXT-FIG. 4.

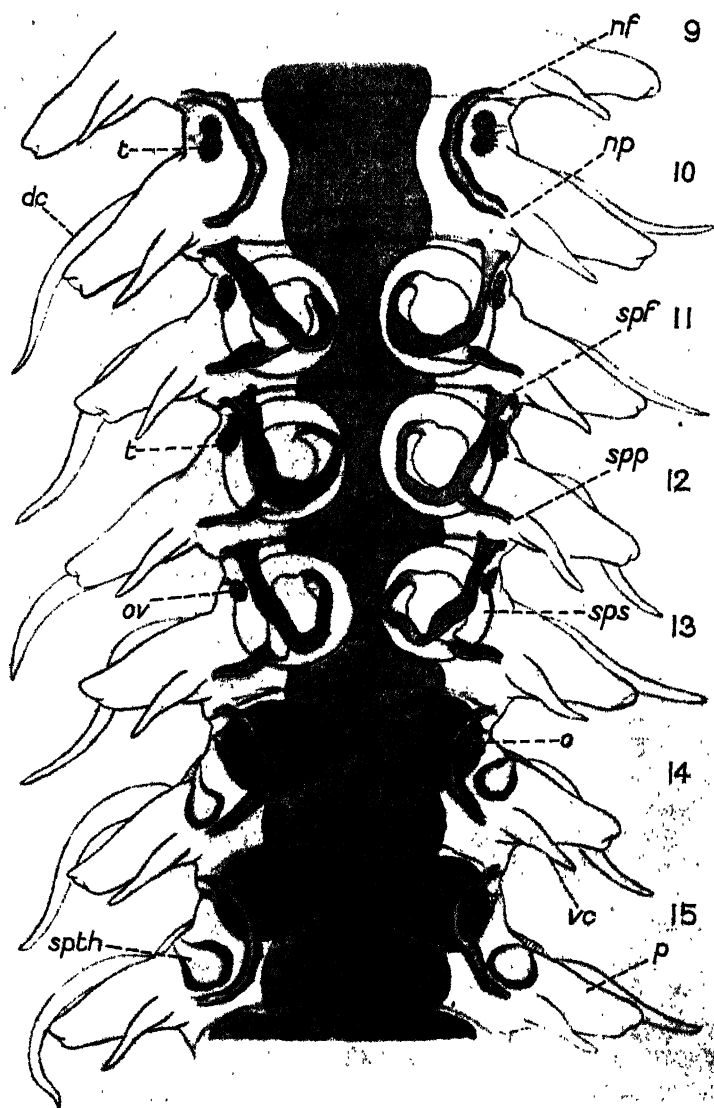


Transverse section of a female segment (14th). *ac*, aciculum; *c*, coelom; *cm*, circular muscles; *dc*, dorsal cirrus; *dlm*, dorsal longitudinal muscles; *dv*, dorsal vessel; *i*, intestine; *nc*, ventral nerve-cord; *nph*, nephridium; *o*, ovum; *p*, parapodium; *spt*, spermatheca containing spermatophores; *vc*, ventral cirrus; *vlm*, ventral longitudinal muscles; *vv*, ventral vessel.

meet in the mid-ventral line. The small undeveloped oocytes then come to lie close to the ventral blood-vessel. In the young ovary the superficial cells merge into the coelomic epithelium which binds it to the body-wall; and when a central oocyte enlarges it bulges into the coelom covered over by a layer of coelomic epithelium, which later forms a thin follicle of flattened cells (Text-fig. 7).

There are no special oviducts, and the nephridia in female segments remain quite unmodified (see below). With their

TEXT-FIG. 5.

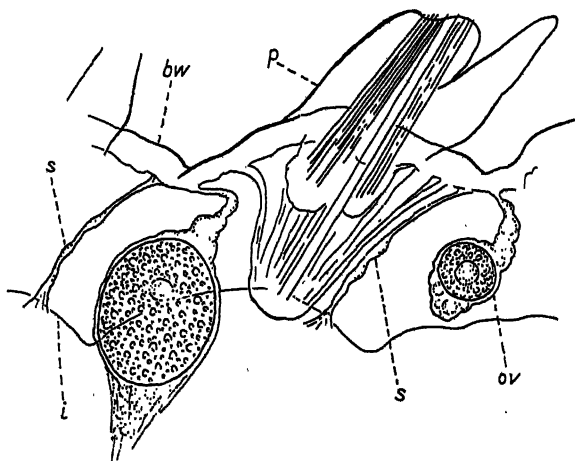


Diagrammatic ventral view of segments 9-15. *dc*, dorsal cirrus; *nf*, nephridiostome; *np*, nephridiopore; *o*, large ovum; *ov*, ovary; *p*, parapodium; *spf*, spermiducal funnel (coelostome); *spp*, spermiducal pore; *sps*, sperm-sac; *spth*, spermatheca; *t*, testis; *vc*, ventral cirrus.

minute nephridiostome they would be incapable of serving as outlets for the large ova.

Unfortunately I was not able to witness the extrusion of the eggs, and the material at my disposal does not allow me to make quite certain of the details of their normal mode of exit. But the preparations seem to show that the eggs escape by bursting through the thin dorso-lateral region of the body-wall. In

TEXT-FIG. 6.



Sketch from life of right side of female segments; ventral view enlarged. *bw*, body-wall; *i*, edge of intestine; *ov*, young ovary; *p*, parapodium; *s*, septum.

transverse sections of worms with young external embryos the body-wall is seen to be deeply infolded between the parapodia; presumably the temporary apertures are quickly closed up.

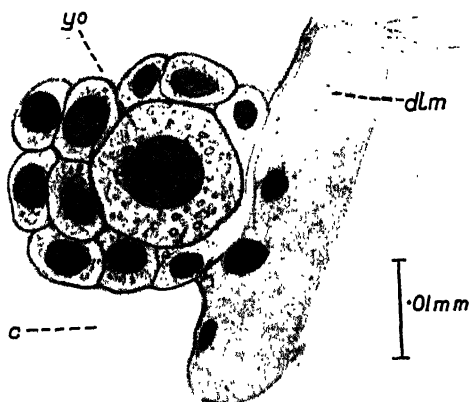
When the ova reach the exterior they are enclosed in a cuticular membrane; within it they undergo development, and it is not till they reach a late stage that they break through the membrane and escape. At first the embryonic shield develops on the convex surface of the egg; but later it becomes curved with ventral surface bent inwards. The embryo remains flexed until many segments and parapodia have formed, and by the

time the young worm breaks through the membrane and straightens out it has acquired almost the general structure of the adult with some eighteen segments.

During this period of growth the young remain attached to the parent in pairs on each female segment by means of special organs of fixation described below.

From what has been said above it is clear that fertilization

TEXT-FIG. 7.

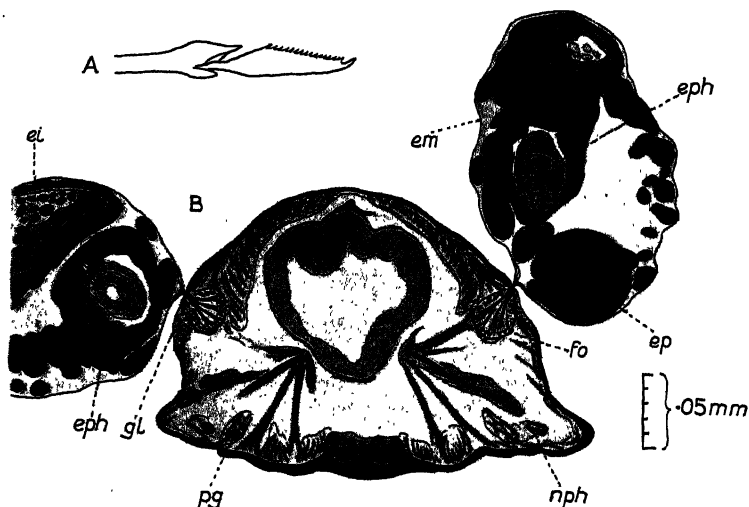


Portion of a transverse section showing a small ovary attached to the dorsal longitudinal muscles, *dlm*. *c*, coelom; *yo*, ovum beginning to enlarge. From a specimen with external embryos.

must take place about the time when the eggs are extruded. It is an interesting fact that in every female segment of an individual with well-developed ova there occurs a pair of spermathecae. The spermatheca is a small sac, blind internally, opening externally by a round pore, and developed as an invagination of the dorso-lateral body-wall just anterior to the base of the dorsal cirrus (Text-figs. 5 and 8). Several of these sacs are generally found containing spermatophores, and it may safely be concluded that the spermatophores have been derived from some other individual, copulation having taken place. Unfortunately I have not been able to observe the process. It is probable, then, that fertilization occurs when the ova are emerging close to the spermathecal pore, and when the

covering membrane is still thin or incomplete. The exact origin of this membrane, in which the external embryo develops, is difficult to make out. Whether it is a vitelline membrane secreted by the egg itself, or a product of the cells of the enveloping follicle, I have not determined for certain; but appearances in sections suggest that it is produced by the egg.

TEXT-FIG. 8.



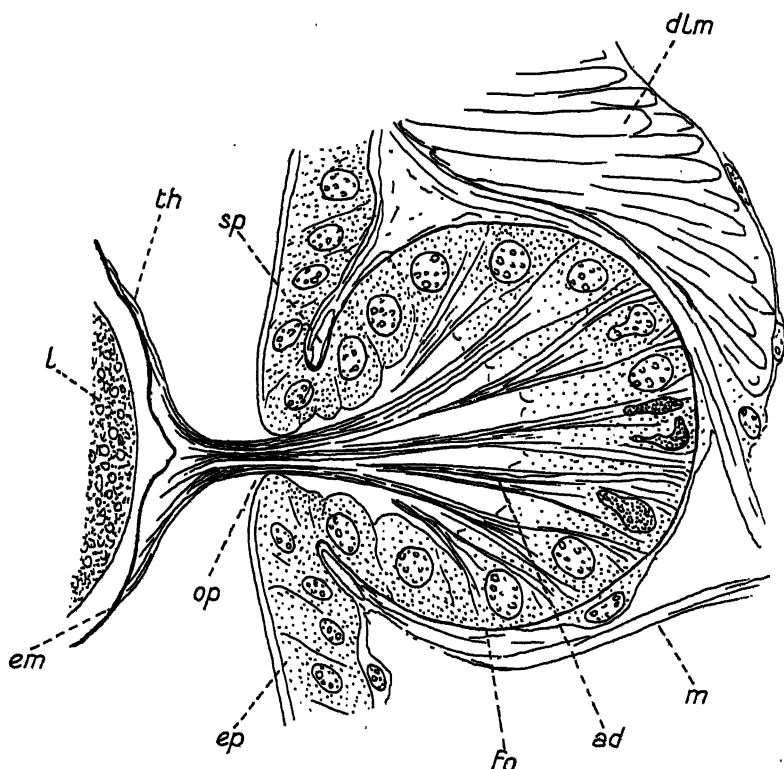
A, enlarged drawing of a compound chaeta. B, transverse section of a female segment with two embryos attached. *ei*, embryonic intestine; *em*, embryonic membrane; *ep*, embryonic prostomium; *eph*, embryonic pharynx; *fo*, organ of fixation; *gl*, glandular area; *nph*, nephridium; *pg*, pedal gland.

The follicle has disappeared by the time the egg has reached the exterior.

Only one pair of ova ripens at a time in each segment, and this takes place almost simultaneously in all the female segments of an individual, though rather sooner in the anterior than in the more posterior segments. When this generation of oocytes has been extruded the ovaries return, so to speak, to the undeveloped condition, and persist as small ovaries attached to the body-wall and longitudinal muscles at the side (Text-fig. 7).

Each, then, contains a central oocyte ready apparently to grow and give rise to a new generation. But I have no observations enabling me to assert that successive generations of young are

TEXT-FIG. 9.



Diagrammatic section of organ of fixation. *ad*, adhesive threads; *dlm*, dorsal longitudinal muscles; *l*, portion of embryo; *em*, embryonic cuticular membrane; *ep*, epidermis; *fo*, organ of fixation; *m*, muscle; *op*, external opening; *sp*, sphincter muscle; *th*, distal ends of threads attached to cuticular membrane.

produced, though this seems highly probable. Similarly, in individuals bearing young the three male segments are seen to have lost all, or nearly all, ripe spermatozoa and spermato-phores, but to retain well-developed testes ready apparently to produce more sperm.

A pair of fixing organs occurs in each of the female segments of embryo-bearing individuals. Each is a spherical sac just anterior to and slightly dorsal of the dorsal cirrus (Text-fig. 8); it is connected with the inner surface of the epidermis, from which it has obviously been derived by invagination. To its coelomic surface are attached some oblique muscle-fibres; while certain muscle-fibres of the circular layer of the body-wall are specialized round its neck to form a sphincter (Text-fig. 9). The cavity of the organ is lined with an epithelium of large cells continuous at the small rounded external opening with the general epidermis. The deeper large cells give rise to numerous delicate fibrils. These are gathered together into sheaves which converge to pass through the opening, and diverge again to spread over the surface of the embryonic membrane (Text-figs. 8 and 9). It is by means of these fibrils that the embryo is firmly attached to the dorsal surface of the segment.

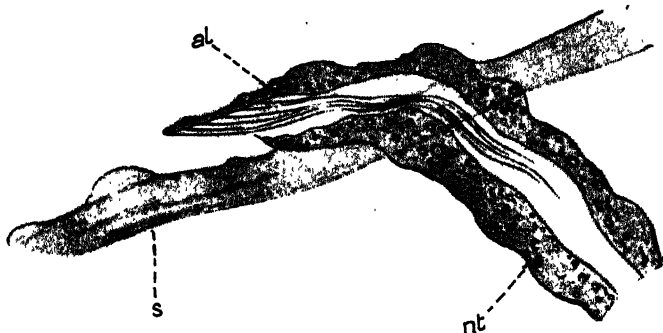
As the fibrils are gathered together to pass through the narrow pore of the organ they form solid-looking rods, some sixteen in number, but they become frayed out again at their distal end. The proximal end of the fibril passes into the substance of the cell and reaches its base. The intracellular portion thus resembles the fibrils often seen in ciliated epithelium passing from the cilia to the base of the cells, and it is possible that the fixing fibrils are modified cilia.

The origin of these highly specialized organs of fixation puzzled me for a long time. They are absent as such in worms with spermathecae and internal ova, but appear fully formed in those bearing external young. It was not till I concluded that the fixing organ must be derived from the spermatheca which occupies the same position, that its mode of development was elucidated. The growth of the organ must be rapid, and my sections do not show a complete series of intermediate stages. Nevertheless, it seems clear that, as the eggs become ready for extrusion, the spermatophores come to lie in the ventral region of the spermatheca and the more dorsal region becomes modified. Here the lining cells acquire the structure

of those of the fixing organ and begin to develop fine fibrils. Later, these cells seem to spread over the whole inner surface of the sac; so that, by the time the spermatophores have been expelled, the whole spermatheca has been transformed into the organ of fixation.

We may now pass to a consideration of the nephridia. A pair of simple nephridia occurs in each segment from the fifth (fourth setigerous segment) to the last, excepting for the eleventh, twelfth, and thirteenth segments, where they are much

TEXT-FIG. 10.

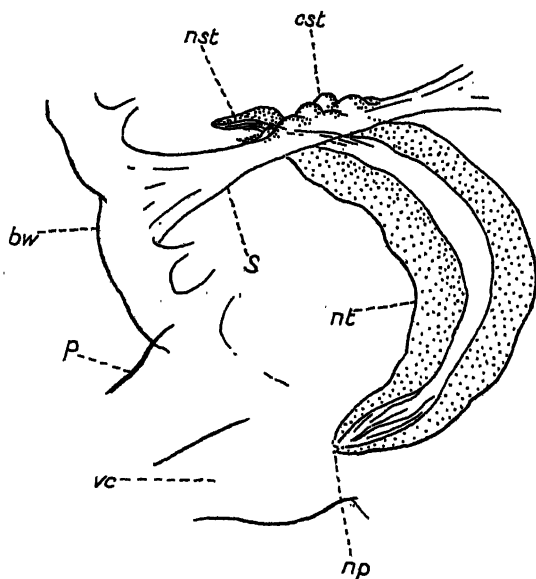


Enlarged view from life of anterior end of nephridium. *al*, anterior lip of nephridiostome; *nt*, nephridial tube; *s*, septum.

modified. The nephridium closely resembles that described by me in an immature *Trypanosyllis* (3). A small open nephridiostome opens into the next segment in front, projecting beyond the delicate transverse septum. The anterior lip of the funnel bears a bunch of long cilia forming a 'flame' which beats down the lumen (Text-fig. 10). The slender tubule with intracellular lumen runs back near the ventral body-wall to a minute external pore situated near the base of the ventral cirrus. These nephridia are difficult to see in the living in spite of the presence of excretory granules in the wall; but, if a little neutral red be added to the sea-water in which the worms are kept, the nephridia stain readily and become clearly visible.

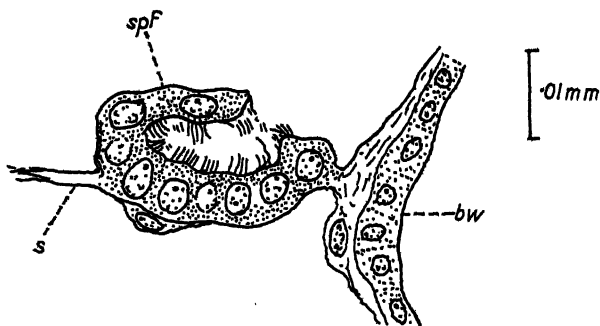
It will be remembered that in the majority of Syllids the

TEXT-FIG. 11.



Sketch from the living young worm of a developing sperm-duct, enlarged. *bw*, body-wall; *cst*, rudiment of coelomostome; *np*, nephridiopore; *nst*, nephridiostome; *nt*, nephridial tubule; *p*, parapodium; *s*, septum; *vc*, ventral cirrus.

TEXT-FIG. 12.



Portion of a transverse section of a male segment, showing a sperm-funnel or coelomostome, *spf*. *bw*, body-wall; *s*, septum.

nephridium becomes converted at maturity in both sexes into a nephromixium by the grafting of a large ciliated coelomostome on to its open extremity, and that these compound nephromixia function as genital ducts (3). The three pairs of sperm-ducts of our *Pionosyllid* are of such mixed origin. The nephromixium is formed early, long before the eggs are full-grown, but young specimens may sometimes be found showing intermediate stages. In Text-fig. 11, sketched from life, may be seen the right nephridium of segment 11; it still has its nephridiostome, but near it a group of coelomic epithelium cells is about to develop into the coelomostome. The wall of the post-septal region of the tubule is also thickened and beginning to become transformed into the sperm-sac, though the characteristic granules have not yet appeared in its cells. In the fully developed sperm-duct the coelomostome is cup-shaped and ciliated; it leads into the nephridium and the nephridiostome can no longer be distinguished (Text-fig. 12).

SUMMARY AND CONCLUSION.

In this paper a new species of Syllid, named *Pionosyllis neapolitana*, is described, whose chief characteristics are that it is hermaphrodite, and has reproductive organs of remarkably complex and constant structure.¹ There are a pair of testes in each of the segments 10, 11, and 12, and a pair of ovaries in every segment from the thirteenth backwards throughout the region of the true intestine. A pair of nephridia with small nephridiostomes occurs in every segment from the fifth backwards, except in segments 11, 12, and 13, in which they become transformed into nephromixia functioning as sperm-ducts. Each sperm-duct is provided with a ciliated

¹ Only once has a variation in the disposition of these organs been found. This specimen has the usual testes and sperm-ducts in segments 10 and 11, but an additional testis on the left side of segment 8, and corresponding sperm-duct in segment 9. The twelfth segment, while possessing the usual testis and sperm-duct on the right, has an ovary on the left. The thirteenth segment has a sperm-duct on the right, and the usual ovary on the left.

coelomostome opening into a male segment, and its post-septal tubule is enlarged into a sperm-sac where the spermatozoa form spermatophores. Presumably copulation takes place, since spermatophores are found lodged in paired spermathecae opening to the exterior on every female segment. One ovum at a time in every ovary enlarges and is extruded dorsally, apparently by breaking through the body-wall. The ova by this time are fertilized. They develop to an advanced stage surrounded by a cuticular membrane, and attached in pairs to every female segment. The young escape from the membrane when about eighteen segments have been formed. When the ova pass to the exterior they become attached to the latero-dorsal surface of the female segments by means of fixing threads formed by special paired organs of fixation. These organs are derived from the spermathecae. Possibly successive generations of ova are extruded, but this has not yet been observed, nor is it known whether the fixing organs can again function as spermathecae. Exactly how and when fertilization takes place has not so far been determined.

DEPARTMENT OF ZOOLOGY AND COMPARATIVE ANATOMY,
UNIVERSITY MUSEUM, OXFORD.

December 11, 1929.

LIST OF REFERENCES.

1. Du Plessis, G.—"Protandrie chez les Syllidiens", 'Rev. Suisse de Zool.', vol. 16, 1908.
2. Fauvel, P.—'Faune de France, 5: Polychètes errantes'. Paris, 1923.
3. Goodrich, E. S.—"Nephridia of the Polychaeta, Part III", 'Quart. Journ. Micr. Sci.', vol. 43, 1900.
4. Haswell, W. A.—"The Exogoneae", 'Journ. Linn. Soc. Zool.', vol. 34, 1918.
5. Pierantoni, U.—"La gestazione esterna", 'Arch. Zoologico', vol. 1, 1903.

On the Cranial Characters of the South African *Brevicipitid, Phrynomerus bifasciatus.*

By

C. G. S. de Villiers, M.A., Ph.D.,

Professor of Zoology, University of Stellenbosch.

With 14 Text-figures.

MUCH work has recently been done on the comparative anatomy and ontogeny of the shoulder-girdle of the Anura, but no attempt has been made to reinvestigate the skull with the aid of modern microtechnical methods ; it is hoped that the present paper will contribute towards the revival of interest in the osteology of the anuran cranium.

The first printed reference to *Phrynomerus* is in Smith (1849), where it was registered as *Brachymerus*, a name which was subsequently found to be preoccupied. A foot-note (Smith's publication has no paging) contains the following information : 'jaws and palate without teeth, tympanum concealed, nostrils close to apex of nose, transverse processes not much extended'. In the general description the following information regarding the cranium is supplied : 'nostril opens forwards and outwards, surrounded by a slightly raised membranous ring. Tympanum partially visible, a little behind the infero-posterior part of the eye'. The drawing of the genus is given on Pl. 43.

Phrynomerus was next figured in Bianconi (1850) and was there (p. 26) called *Dendrobates inhambanensis*. The plate (Pl. V, fig. 4) leaves no doubt that *Phrynomerus bifasciatus* is the actual animal referred to. No important cranial characters are mentioned, except that the tympanum is stated to be present. Günther's catalogue (1858) relegates *Phrynomerus* (= *Brachymerus*, Günther) to the family *Brachymeridae* among his series of frogs 'without maxillary teeth and with perfectly developed ear'. Only one osteological

character of the family is stressed: the 'dilated processes of sacral vertebra' (p. 124). Under the definition of the genus, the following skeletal features are mentioned: 'teeth none in jaws nor in palate nor a sharp bony ridge. Tympanum hidden; Eustachian tubes very small. Sacral vertebrae not much extended'. The name *Phrynomantis*, by which *Phrynomerus* was generally known until recently, was proposed by Peters (1867). This publication as well as three of four others by the same author and by Cope are not available in South Africa. Peters again discusses the genus in the *Reise nach Mosambique* (1882); the classification adopted is *Bufoformia-Engyostomata-Phrynomantis* (p. 172). The first two groups are not defined. Cranial and other skeletal characters mentioned under the description of the genus *Phrynomantis* are: 'Kiefer schwach, ebenso wie der Gaumen zahnlos. Trommelfell wohl entwickelt, aber unter der Haut versteckt. Sternalapparat ohne Manubrium und ohne Claviculae, wie bei *Diplopelma* und *Systema*; das stielförmige Sternum trägt einen sehr grossen breiten Xiphoidknorpel.' On Pl. IX, figs. ix and ix a, drawings are given of the ventral portion of the shoulder-girdle, of the expanded transverse processes of the ninth vertebra, and of the pelvic girdle. Boulenger (1882) adopts (p. 172) the name *Phrynomantis*, which he classes under *Engyostomatidae* (p. 146), characterized by the following cranial characters; absence of maxillary teeth and of the fronto-parietal fontanelle. The genus itself (p. 172) is stated to lack vomerine teeth and to possess a cutaneous fold across the palate between the choanae. The obscurity of the tympanum is also referred to.

Boulenger's 'Revised List' (1906-9) mentions no cranial characters of *Phrynomerus*, and the *Engyostomatidae*, under which family the genus is classified, are not clearly defined; they are stated to possess 'no teeth'. Hewitt (1911) quotes Werner (1910) in remarking upon the possible affinities of *Cacosternum* and *Phrynomerus*. The general depression of the head and body in both genera seemed to him an unreliable criterion. The life-history was described by Power (1926) and Wager (1926); which latter author originally referred

the larvae to *Hyperolius* (p. 174). Additional data regarding the development were afforded by Power (1927, p. 415), Loveridge (1925), and Wager (1929). Hewitt had already in 1919 regarded the affinity of *Phrynomerus* and the *Anhydrophryne-Cacosternum* group with suspicion, since it seemed to be based on the characters of the shoulder-girdle solely. Noble's important work of 1922 asserts (p. 19) the affinities of *Cacosternum*, *Anhydrophryne*, and *Phrynomerus*; and the toothed *Dyscophidae* and the Old World *Brevicipitid* forms are grouped (p. 20) into a new family, the *Brevicipitidae*. Power (1927, p. 250) calls attention to the similarity of the larvae of the Indian *Microhyla* and the South African *Phrynomerus* and to the absence of any specialization in the larvae of *Cacosternum*. Noble (1926, p. 19), after comparing specimens of '*Phrynomantis*' from the East Indies with African species, came to the conclusion that they are generically distinct and proposed the new genus *Phrynomerus* for the African species (p. 20). In his well-known work on the frogs of the Congo (1924) Noble had already expressed the opinion (p. 278) that the East Indian and African forms were not congeneric. Nieden (1926) gives no new information about *Phrynomerus* (*Phrynomantis*, p. 12). Under *Engyostomatidae* (p. 1) the epicoracoids are stated to be fused. *Phrynomerus* is grouped under the sub-family *Engyostomatinae* in which both jaws are toothless, although vomerine teeth are represented in some genera. Noble (1926, p. 4), remarking upon the dissimilarity of the larvae of *Cacosternum* and *Phrynomerus*, comes to the conclusion (p. 4) 'that the genera have descended from totally different stocks'. Noble (1927) enumerates the main peculiarities of the *Dyscophid-Brevicipitid* larva (p. 114), but notes that the larva of *Cacosternum* does not conform to this type. *Hemisus* has larvae also differing from those of *Brevicipitid* tadpoles (Wager, 1927) in the possession of horny teeth and a sinistrally situated spiracle, so that it may possibly have to be removed from the *Brevicipitidae*; it owes its inclusion in this family mainly to certain superficial resemblances to *Breviceps*.

MATERIAL AND TECHNIQUE.

The adults and tadpoles of *Phrynomerus bifasciatus* were very kindly supplied by Mr. J. H. Power of Kimberley, who collected the material at Lobatsi. Alcoholic preservation was employed. Two stages were examined: the young, fully metamorphosed froglet and the adult. The specimens were carefully skinned and placed in water to remove the alcohol. This is best accomplished by placing the glass container in a thermostat kept at about 50° C. and changing the liquid every half-hour, until all alcohol has been washed out. The entire froglet and the head of the adult were then decalcified in Ebner's solution (see Mayer, 1920) as supplied by Dr. Grübler's laboratory. Five days proved to be sufficient for the froglet, whereas the head of the adult was decalcified for nine days. The tissues were in no wise deleteriously affected, Ebner's solution being an extremely safe decalcifier. The objects were bulk-stained in Haemalum and thoroughly differentiated with alum solution of 4 per cent. They were then embedded in paraffin in the usual way. It was to be expected that great mechanical difficulties would be encountered in preparing sections of a large, hard object like the Anuran skull. This, fortunately, did not prove to be the case. Contrary to the usual advice of microtomists, I embed large, hard objects in fairly soft paraffin of a melting-point of approximately 40° C., and succeed in obtaining perfect series. For a counterstain van Gieson may be used, with or without Lichtgrün. But since the picric acid in van Gieson tends to bleach the nuclear stain, Fuchsin alone may be employed. Cartilage, connective tissue and decalcified bone are quite satisfactorily differentiated by means of this process. Bismarck Brown, the finest Cartilage stain known, was not used, as it does not readily stain in alcoholic solution and it was not deemed advisable to take the slides through to water, and thereby taking the risk of the sections washing off.

THE SKULL OF PHRYNOMERUS BIFASCIATUS.

The details of the anatomy of the Anuran skull are best studied in Ecker-Wiedersheim-Gaupp, erste Abteilung, 1904.

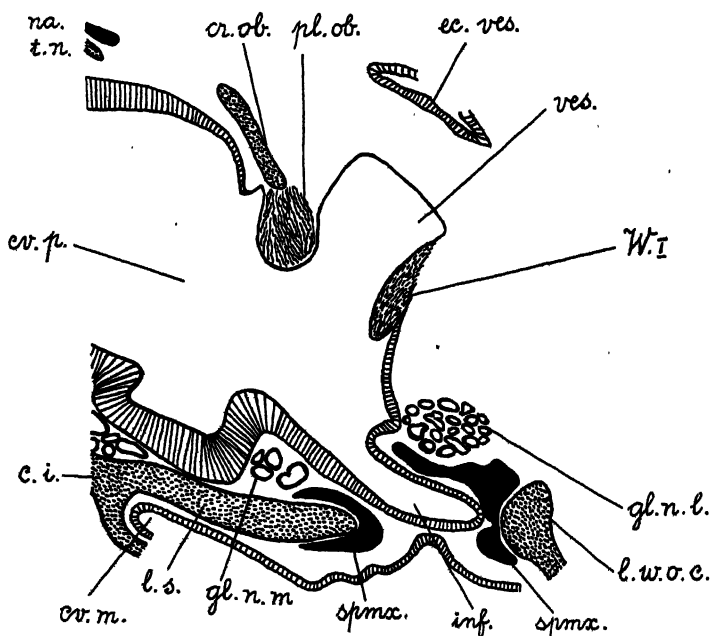
The true homology of the paraquadrate (= 'Squamosum') and the quadratomaxillary (= quadratojugal) were first elucidated by Gaupp (1894). The development of the skull may be studied in Gaupp (1893) and (1906). Kothe reinvestigated the ontogeny of the auditory cartilages in 1910. Histological details of the sense organs are furnished by Krause (1923). For the study of the anatomy and development of the Anuran skull, the Ziegler models, based on Gaupp's reconstructions, are absolutely indispensable. The species investigated is the European *Rana fusca*.

The olfactory capsule.

The olfactory region of the Anura is notorious for its complexity, particularly at the level of the external narial aperture. *Phrynomerus* possesses both pre-nasal cartilages, as well as the alary, which latter occupies the lateral wall of the vestibule, whereas its roof is strengthened by the tectum nasi in the anterior region and the oblique cartilage in the posterior. The vestibule has much the same structure as in *Rana fusca*, but the plica obliqua is thick and blunt, whereas it is elongated in *Rana*. In *Phrynomerus* it is, moreover, more posteriorly situated, as it only appears in section after the closing of the narial aperture; it is suspended from the cartilago obliqua, not from the tectum nasi as in *Rana* (see Text-fig. 1). Gaupp (1904, p. 624) describes two 'Wülste' in connexion with the vestibule; one occupies the place of the alary cartilage when it disappears, and the other is finger-shaped and lies in the posterior angle of the anterior narial aperture. They are both represented in *Phrynomerus* (Text-figs. 1 and 2). An important difference between *Rana* and *Phrynomerus* concerns the absence of the recessus sacciformis in the latter genus. The anatomical details of the structure are described by Gaupp (1904) on pp. 625 and 633, from which it appears that the recess is a mucous sac underlying the 'Wulst', which takes the place of the alary cartilage, and communicates with the niche-like posterior division of the vestibule, with the infundibulum and with the cavum medium. Distinct vestiges of

the vestibular and infundibular portions of the recessus are present, but the cavum medium shows no traces of such evaginations (Text-fig. 2). The details of the anatomy of the following organs are exactly as described in Gaupp (1904);

TEXT-FIG. 1.

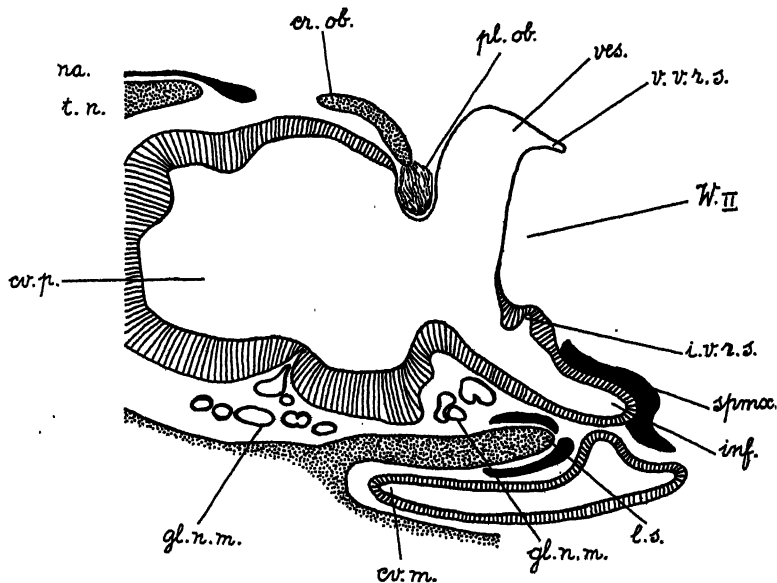


Transverse section through the vestibular region of the nose. *c.i.*, crista intermedia; *cr.ob.*, cartilago obliqua; *cv.m.*, cavum medium; *cv.p.*, cavum principale; *ec.ves.*, external ectoderm covering the vestibule; *gl.n.l.*, glandula nasalis lateralis; *gl.n.m.*, glandula nasalis medialis; *inf.*, infundibulum; *l.s.*, lamina superior; *l.w.o.c.*, lateral wall of olfactory capsule; *na.*, nasal; *pl.ob.*, plica obliqua; *spm.*, septomaxillary; *t.n.*, tectum nasi; *ves.*, vestibule; *W.I.*, finger-shaped 'Wulst' of Gaupp.

glandula nasalis medialis, glandula nasalis lateralis, glandula intermaxillaris; cavum principale, cavum inferius, recessus medialis, recessus lateralis, infundibulum, pars communis cavi mediani et cavi inferioris and the communication of the ductus lacrimalis with the cavum medium.

The region of the choana is, however, considerably different from that of *Rana*. The following statement occurs in Boulenger, 1882, p. 172: 'a cutaneous fold across the palate between the choanae'. Nieden (1928) also mentions this peculiar feature of *Phrynomerus* and unquestionably derived the

TEXT-FIG. 2.

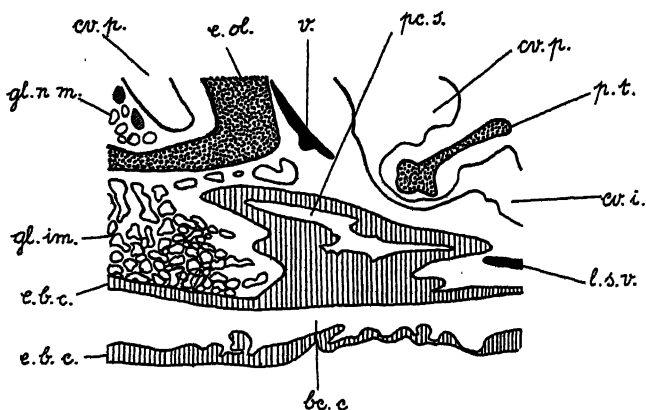


Transverse section through the vestibular region of the nose.
i. v. r. s., infundibular vestige of recessus sacciformis; *v. v. r. s.*,
 vestibular vestige of recessus sacciformis; *W. II.*, larger 'Wulst'
 of Gaupp. Other abbreviations as in Text-fig. 1.

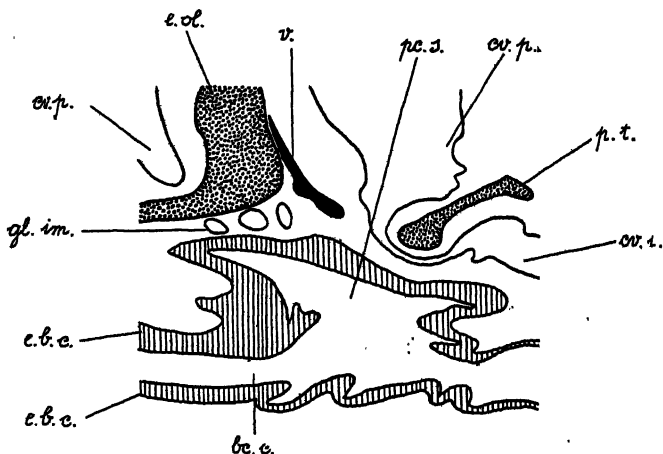
information from Boulenger's catalogue, as the phrase is literally translated into German. The histological details shown by transverse sections have revealed a most remarkable state of affairs, which may prove to be of great morphological interest.

The 'fold' mentioned by Boulenger is first noticeable in transverse sections as two patches of tangentially cut epithelium lying above the epithelium of the oral cavity and of a similar histological structure. They are bounded medially by the glandula intermaxillaris and laterally by the maxillary bone.

TEXT-FIG. 3a.



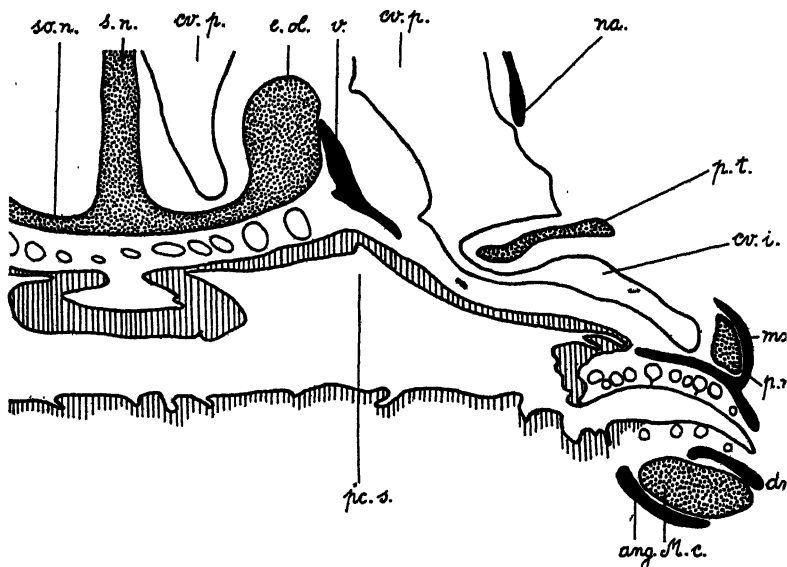
TEXT-FIG. 3b.



Consecutive transverse sections through the prechoanal sacs. *ang.*, angular; *bc.c.*, buccal cavity; *cv.i.*, cavum inferius; *dn.*, dentary; *e.b.c.*, epithelium of buccal cavity; *e.ol.*, eminentia olfactoria; *gl.im.*, glandula intermaxillaris; *l.s.v.*, lateral squame of vomer; *M.c.*, Meckel's cartilage; *mx.*, maxilla; *pc.s.*, prechoanal sac; *p.m.*, processus maxillaris anterior; *p.t.*, planum terminale; *s.n.*, septum nasi; *so.n.*, solum nasi; *v.*, vomer. Other abbreviations as in previous figures.

The epithelial patches next acquire lumens communicating with one another (Text-fig. 3 a) and the common sac opens into the oral cavity (Text-fig. 3 b). The oral openings of the right and left sacs are separated by a broad barrier of thickened epithelium of the buccal cavity, which however becomes rapidly attenuated (Text-fig. 3 c) and finally disappears. The anterior

TEXT-FIG. 3c.



borders of the two evaginations, together with the short, conic tongue-like barrier, form the transverse band or fold referred to by Boulenger and Nieden. But it is not situated between the choanae, but in front of them, for as will be seen from Text-fig. 3 c the choanae have not yet appeared in section. The hinder margin of the 'fold' is separated by a thickness of forty-seven sections from the anterior rim of the choana itself! It is more than probable that the sacs referred to above are vestiges of the organ of Jacobson. Gaupp (1904) homologizes the recessus medialis of the cavum inferius, which is a thick-walled pouch situated on the side of the septum and between

the solum nasi and the crista intermedia, with the organ of Jacobson. In Amniotes the organ develops a buccal opening which is however absent in Amphibia. Professor Ivar Broman of the University of Lund has an important paper (1919) on the organ of Jacobson, which he has kindly sent me. Unfortunately his researches were confined to the Amniotes. In mammals Jacobson's organ possesses its own glands and erectile tissue, both of which were found to be absent in the Reptiles investigated. The buccal sacs described above for *Phrynomerus* do not possess their own glands, but are in close proximity to the glandula intermaxillaris. No innervation of any kind could be made out, although Jacobson's organ is supposed to be innervated by the first and fifth cranial nerves. Broman maintains that the organ of Jacobson contains no air, but sucks liquid from the buccal and narial cavities and may be considered as a portion of the narial apparatus persisting in the piscine condition. He concludes by ascribing a 'Wassergeuchsfunktion' to the organ. If the primitive land Anamniotes possessed a buccal division of Jacobson's organ, *Phrynomerus* represents the only known form possessing a vestige of it. The posterior division of the olfactory capsule is remarkable for the enormously developed eminentia olfactoria, which in the frog consists of a low, broad ridge on the floor of the cavum superius medial to the choana (see Gaupp, 1904, fig. 145). In *Phrynomerus* (see Text-fig. 4) the mucous membrane of the eminentia is spread over a supporting axis of cartilage, representing an upgrowth of the solum nasi. This cartilaginous axis is covered laterally by the dorso-ventrally elongated portion of the vomer to be described below, and begins to appear in section at the niveau of the planum terminale of the cartilago obliqua. It decreases in height, but increases in breadth posteriorly and possesses a laterally directed spine postchoanally. Before finally disappearing, some distance anterior to the foramen olfactorium, the high narrow type of cross-section is again met with in the eminentia. The cartilage is then surrounded by sectioned branches of the olfactory nerve. The comparatively large eminentia of *Phrynomerus* has the effect of increasing

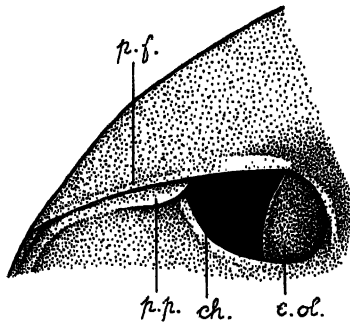
the olfactory surface, resulting in an enhanced olfactory function, probably of great importance in a land form.

Membrane bones of the olfactory region.

The olfactory capsule is invested in the Anura by the premaxilla, the maxilla, the vomer, and the septomaxillary. The palatine in reality invests the ventral surface of the antorbital process, but may be conveniently treated here as well.

The premaxilla in the adult *Phrynomerus* is entirely

TEXT-FIG. 4.

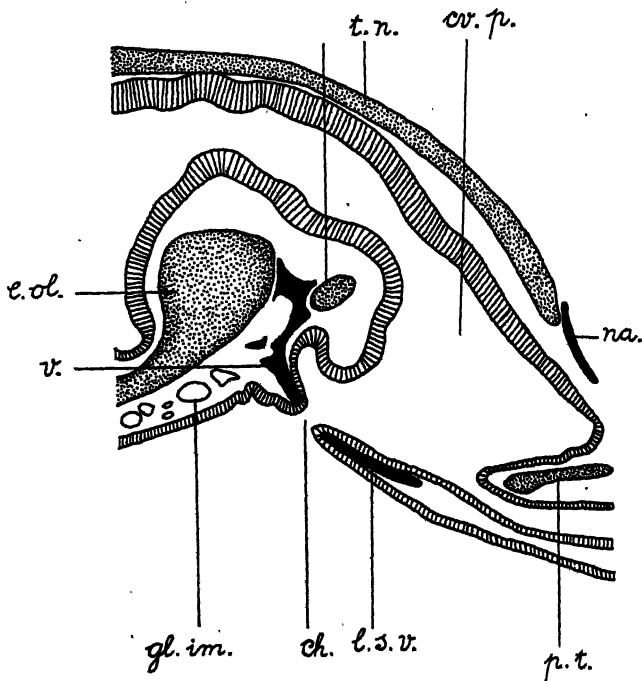


Schematic drawing of the right choana. *p.f.*, palatal fold (Gaumenleiste); *p.p.*, plica palatalis. Other abbreviations as in previous figures.

edentulous. It consists, as in the frog, of a body, supported by the cartilago prenasalis inferior, and possesses three bony outgrowths: (a) a dorso-ventral blade supported by the cartilago prenasalis superior, and (b) and (c) two palatal squames investing the solum nasi, but in reality separated from it by the glandula intermaxillaris. The maxilla is also edentulous; it is supported partially by the crista subnasalis and partially the processus maxillares and the processus pterygoideus. But whereas in the frog the maxilla articulates directly with the outer palatal squame of the premaxilla, the two bones are separated in *Phrynomerus* by a thin, strip-like cartilage. In sections, this cartilage will be seen to represent a retrally flexed crista subnasalis, which normally (e.g. in the frog) would

not be visible in a palatal view, and is, moreover, short and blunt instead of long and rod-like. It finally disappears from sections at the level of the anterior limits of the cartilaginous axis of the eminentia. The maxilla articulates with the quadratamaxillary posteriorly, in such a way that the anterior tip of the latter bone

TEXT-FIG. 5.



Transverse section through the anterior portion of the choana.
Abbreviations as in previous figures.

lies craniad to the posterior tip of the latter. The vomer is comparatively large and quite toothless in the adult as well as in the young; anteriorly it invests the lateral margin of the solum nasi, extending dorsally along the cartilaginous axis of the eminentia, but soon a short medial and a long lateral squame make their appearance; the former is much shorter than in the frog, so that the vomer appears to lie on the side of the

choana. The dorso-ventral squame of the vomer is clamped by an anteriorly directed process of the lateral margin of the solum nasi seen in Text-fig. 5, cut through the choanal aperture. It would appear from the drawing as though the choana pierces the lateral squame of the vomer; this is due to the fact that the bone fringes the anterior and medial margins of the choana. Towards the hind end of the choana, the vomer is synostotically fused with the extremely short palatine, which is a trough-like bone, the groove of which is applied to the anterior margin of the processus antorbitalis. Noble (1926) figures the vomers of *Phrynomerus* on p. 20; each bone is traversed by a transversely situated foramen which divides it into a large anterior and a small posterior portion. Judging from microtomed series, the bone figured by Noble (loc. cit.) is not a vomer but a vomero-palatine. In his work on the Brevicipitid toads of Madagascar ('Am. Mus. Nov.', no. 232, 1926 b) the following anatomical details of the vomer of the Malagasy forms are given (p. 2): 'The vomer of each side is divided into an anterior and a posterior half. The posterior of each side usually overlies the palatines and has been confused with these latter bones.' It is very likely that the Malagasy Brevicipitids also possess a vomero-palatine as *Phrynomerus* undoubtedly does. If this is the case, the toothed Malagasy Brevicipitids would be very closely allied to the Ethiopian *Phrynomerus*, which therefore represents an extremely interesting relic of the original Ethiopian fauna, at a time when Madagascar was still a part of the African continent. It is very remarkable that Noble should nowhere mention the absence of palatines in *Phrynomerus*, for if he considers the vomero-palatine as a split vomer, what has become of the palatines? In concluding these remarks on the vomer, it should be mentioned that according to Broom (1902 and 1915) *Amphibia* possess a premaxilla (= vomer) and a vomer (= parasphenoid). Embryologists are likely to accept this view much less readily than palaeontologists; the older theory, provisionally accepted also by Versluys (1924), lays more stress on the parts of the chondrocranium these bones invest, than on their comparative topography.

The septomaxillary is first encountered in sections as a bony bridge between the lateral tips of the two laminae of the crista intermedia. Immediately behind the bone the infundibulum communicates with the cavum medium; the two cavities are shown in close proximity in Text-fig. 2. The septomaxillary may justly be considered as a membrane bone primarily of the lamina superior cristae intermediae, which is its main support. It terminates posteriorly in front of the planum terminale of the cartilago obliqua.

Much importance of a phylogenetic and systematic nature has been attached by Noble (1926 a) to the relations of the nasals to the olfactory capsule. In the *Cacosternum*-*Anhydrophryne* group the nasals leave much of the tectum nasi exposed, so that the os en ceinture appears on the dorsal surface between the nasals. *Phrynomerus*, however, has the Ranid type of the arrangement of the nasals, as will be described below. The anterior tip of the nasal appears in section between the tectum nasi and the cartilago obliqua, but soon comes to rest upon the tectum (see Text-fig. 2), in such a fashion that the mid-dorsal portion only of the latter is exposed. Laterally the nasal never extends over the planum terminale; this is also the case in *Rana*. That part of the bone projecting beyond the tectum is considerably thickened. The posterior margin has a bay as in the frog, but appreciably deeper. An articulation of the nasal with the nasal process of the maxilla does not occur, although it may usually be seen in Ranidae. It is important to note that the nasal disappears from section before any ossification is met with in the chondocranium, proving that the enlarged os en ceinture bounded laterally by the nasals does not occur in *Phrynomerus*.

Ossifications in the sphenethmoid region.

The single cartilage bone (os substitiens), present in this region of the *Anuran* skull, is sometimes termed the orbitosphenoid. The designation sphenethmoid is however preferable, since the ossification not only takes place in the dorso-ventrally situated trabecular derivative, but spreads on to the region of the

olfactory foramen and even the medial portion of the processus antorbitalis. It is perhaps best to use Cuvier's nomenclature, *os en ceinture*, adopted by Dugès and even by modern authors like Versluys (1924, vol. ii, p. 312). This designation also expresses the girdle-form of the bone, one of its chief characteristics; for it also extends ventrally, to include part of the solum nasi and of the base of the skull in the region of the tips of the parasphenoid. In *Phrynomerus*, however, the mid-ventral

TEXT-FIG. 6.

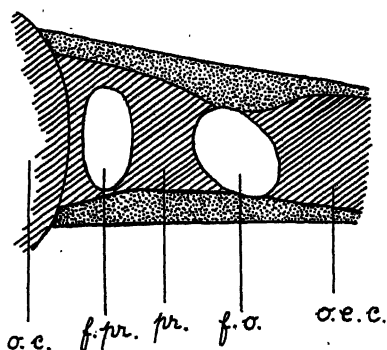


Diagram of the right side of the cranium. *f.o.*, foramen opticum; *f.pr.*, foramen prooticum; *o.c.*, otic capsule; *o.e.c.*, *os en ceinture*; *pr.*, prootic.

region of the base of the skull remains cartilaginous, so that the *os en ceinture* is not in the form of a girdle, but distinctly paired. Moreover, the dorsal rim of the side of the skull is not ossified, so that the fenestra dorsalis is laterally bounded by cartilage. The *os en ceinture* in *Rana* does not reach the optic foramen, which is entirely surrounded by cartilage. In *Phrynomerus*, however, the *os en ceinture* forms the anterior, and the prootic the posterior boundary of the foramen, so that cartilage is represented mid-dorsally and mid-ventrally only. This condition is schematized in Text-fig. 6.

The single ossification of the Anuran otic capsule is termed the prootic, but in reality this bone also extends to the side of the brain-case and forms the posterior boundary of the optic

foramen as described above. The relations to the capsule in the frog are as follows: the bone forms the posterior margin of the foramen prooticum and extends on to the plate separating the brain-case from the cavity of the capsule, stopping short at the middle of the foramen acusticum, whose anterior margin is therefore formed by the prootic. Posteriorly the bone is continued as (a) a dorsal ossification of the capsule, and (b) a lateral ossification lying ventral to the crista parotica, but not reaching the fenestra ovalis. In *Phrynomerus* these two ossifications are entirely absent, but the septal ossification is similar to that of the frog. It would therefore appear that in the former animal the bone is more strongly developed in an anterior than in a posterior direction.

The occipital region has in recent *Amphibia* been assumed to possess an exoccipital ossification only, but at the last Anatomical Congress at Tübingen, Stadtmüller proved that a basioccipital is present in some *Urodeles*. The exoccipital of *Phrynomerus* lies beyond the foramen endolymphaticum, which is surrounded by cartilage as in the frog. The posterior margin of the foramen acusticum is the anterior septal limit of the exoccipital, which also entirely surrounds the foramen jugulare. Ventrally a tract of unossified planum basale persists between the exoccipitals; dorsally the two exoccipitals do not touch each other but are separated by the triangular cartilaginous tectum synoticum. No traces of the notochord persist in the planum basale.

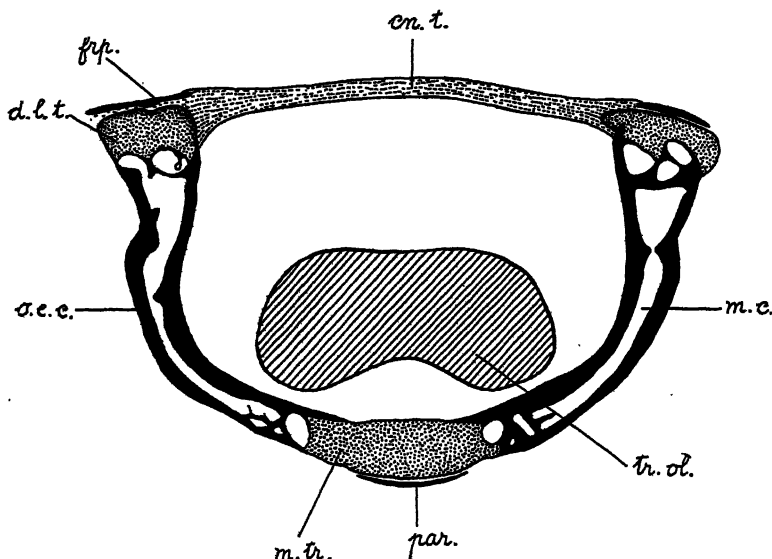
The roof of the chondrocranium is considerably different from that of the frog, in which the fenestra dorsalis is bounded posteriorly by the median and the transverse taeniae. These are entirely absent in *Phrynomerus*, as in the small South African *Ranid* *Arthroleptella*. The fenestra frontalis is therefore confluent with the fenestra parietalis, a condition which represents a further reduction of the cranial cartilaginous roof. Such a composite fenestra is best termed a fenestra parieto-dorsalis.

Membrane bones of the neurocranium.

The bones of the secondary upper jaw and the nasals and septo-maxillaries were discussed above. The parietals fuse in all *Anura* with the frontals to form the two fronto-parietals, which are closely apposed and cover the taeniae and the two dorsal fenestrae. Some authors attach great morphological importance to what is described as a fronto-parietal fontanelle or foramen. Boulenger (1882) finds this fontanelle absent in all Engyostomatidae. Hewitt (1911) states that a fronto-parietal 'foramen', not very felicitously expressed, is present in *Cacosternum*. The fact that Smith (1849) figures a similar foramen for *Breviceps*, was not considered by Hewitt (1919) as a reliable criterion of mutual affinity. The fontanelle in question is presumably that portion of the fenestra frontalis left exposed by the divergent anterior tips of the fronto-parietals, and is typically present in *Rana*. In *Phrynomerus* the cartilaginous roof of the chondrocranium representing the anterior boundary of the fenestra dorsalis is not represented or very feebly so; on its lateral vestiges the anterior tips of the fronto-parietal rest. The roof of the fenestra is constituted by thick fibrous tissue, in which the narrow fronto-parietals are imbedded. Text-fig. 7 represents a section through the anterior region of the skull showing an enormous gap, persisting between the fronto-parietals even in the region of their maximal development (Text-fig. 8). *Phrynomerus*, therefore, has an enormous fronto-parietal fontanelle, not restricted to the sphenethmoid region, but present right up to the tectum synoticum. The brain is protected by the connective tissue filling up the fontanelle, which is macroscopically not easily discernible, but is clearly revealed in sections stained with fuchsine or van Gieson. The parasphenoid has the same relations to the chondrocranium as in the frog. The pterygoid invests the inner surface of the processus pterygoideus in the subocular region, but in the niveau of the processus quadratus very remarkable conditions obtain, which necessitate the pterygoid, paraquadratus, and quadratimaxillary being discussed together.

Some preliminary morphological and osteogenic considerations are however necessary. In the first place, the 'squamosal' (or 'tympanic', Gaupp, 1904) is not in living Amphibia an investing bone of the otic capsule, but of the processus quadratus and

TEXT-FIG. 7.

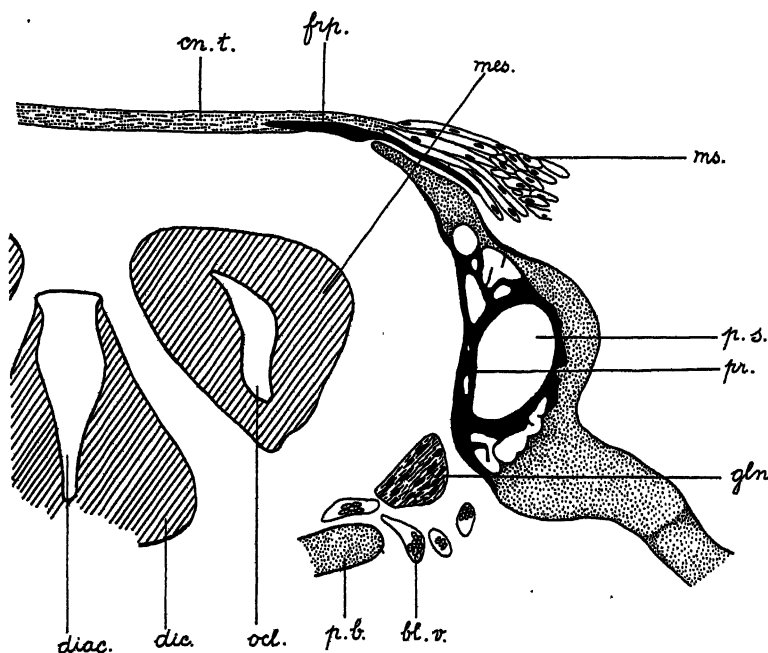


Transverse section of the skull in the region of the os en ceinture. *cn. t.*, connective tissue; *d. l. t.*, dorsal limits of the trabecula (cartilaginous); *frp.*, fronto-parietal; *m. c.*, marrow cavity; *m. tr.*, median ventral cartilaginous trabecular derivative; *par.*, parasphenoid; *tr. ol.*, tractus olfactorius. Other abbreviations as in previous figures.

of the crista parotica, which is not solely a derivative of the otic capsule but also of the palato-quadratum. It is therefore obvious, as Gaupp very clearly demonstrated in his famous paper in Schwalbe's 'Morphologische Arbeiten' of 1894, that Parker's squamosal is not represented in living Amphibia, but that the bone so called, and also represented in Stegocephalia between the true squamosal and the quadratomaxillary, should be termed a paraquadrato. The homology of the 'quadratojugal' was also elucidated by Gaupp in the same paper and its development was

discussed in the articles of 1892 and 1906. Gaupp decided to call this bone a quadratomaxillary, a nomenclature that has been adopted in most modern works on comparative osteology, although palaeontologists have in the main kept to the older

TEXT-FIG. 8.



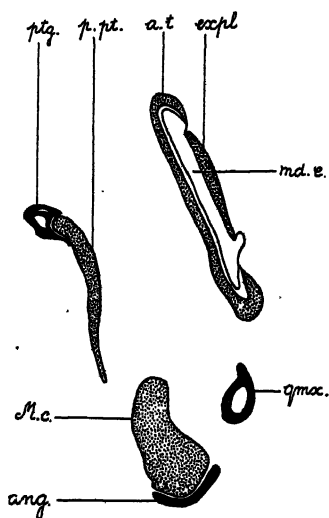
Transverse section through the anterior limits of the inner ear. *bl.v.*, blood-vessels; *diac.*, diacoele; *dic.*, diencephalon; *gln.*, ganglion in the foramen prooticum; *mes.*, mesencephalon; *ms.*, muscle; *ocl.*, optocoele; *p.b.*, planum basale; *p.s.*, perilymphatic cavity. Other abbreviations as in previous figures.

nomenclature. It is also necessary at this juncture to interpolate a few remarks on osteogenesis in general. Gaupp's ossa substituentia and ossa investientia are now universally recognized as skeletal elements derived from totally different skeletogenous strata and are phylogenetically quite distinct. It is now also known that membrane bones are, in their early ontogeny at least, separated from the cartilage they invest by a connective

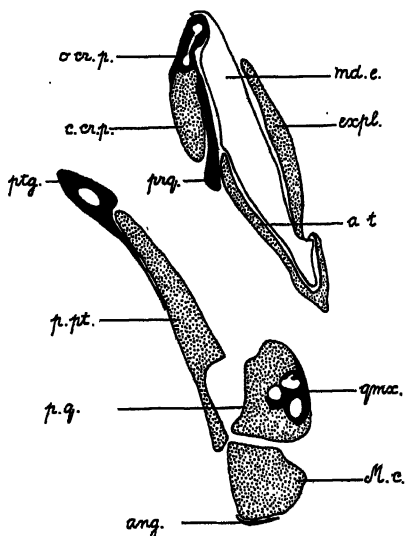
tissue membrane, which may however disappear, so that the membrane bone simulates perichondrial ossification of the cartilage. Or the membrane bone may invade the cartilage, so that a mixed bone results. In the procoracoid of *Xenopus* (de Villiers, 1929) the cartilage ossifies, in spite of the connective tissue separating it from the investing clavicle persisting. Another interesting variant is the ossification of the quadrato-maxillary of the frog, which according to Gaupp starts as an ossification of the ligamentous material, external to the chewing-muscles, then invades the processus quadratus and gives rise to a mixed bone originating partly as a sesamoid ossification and partly as an os substitiensi. Gaupp's results are entirely borne out by my own researches (in the press) on the ossification of the processus quadratus of *Arthroleptella*.

The relations of pterygoid, paraquadrato, quadrato-maxillary and associated cartilages are best understood by reference to the series of drawings, *a-c*, given in Text-fig. 9. It should be explained that the reason why the otic capsule does not appear in section is due to the elongation of the crista parotica and processus oticus palatoquadrati, which is again bound up with the remarkably anterior position of the suspensorium in *Phrynomerus*. In Text-fig. 9 *a* the processus pterygoideus is rapidly passing from the circular type of cross-section met with in the subocular region to the dorso-ventrally elongated type, as the section is cut in the transitional region between the processus pterygoideus and the processus quadratus. The pterygoid invests the medio-dorsal surface of the processus pterygoideus from which it is separated by connective tissue. The bone possesses well-marked marrow cavities, constituting a useful but not conclusive criterion of the identity of a membrane bone as distinguished from perichondrial ossification of the invested cartilage. Meckel's cartilage is invested ventrally by the angular (= goniale = dermarticulare). The quadrato-maxillary has a lateral position between the annulus tympanicus and Meckel's cartilage, is entirely osseous and possesses a central marrow cavity. In the succeeding sections cartilage appears on the inner surface of the bone; this is of course the

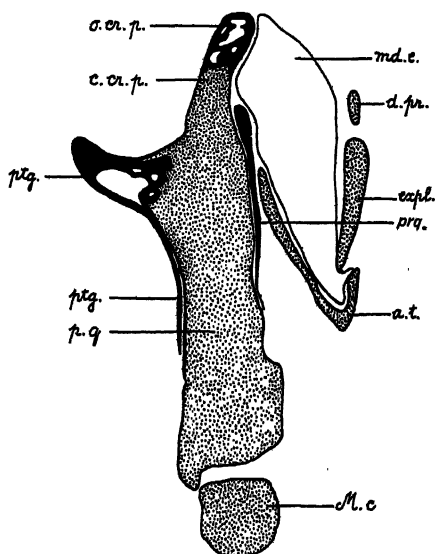
TEXT-FIG. 9a.



TEXT-FIG. 9b.



TEXT-FIG. 9c.



Consecutive transverse sections through the suspensorial region. *a.t.*, annulus tympanicus; *c.cr.p.*, cartilaginous portion of crista parotica; *d.pr.*, dorsal process of extraplectral; *expl.*, extraplectral; *o.cr.p.*, ossified portion of crista parotica; *p.pt.*, processus pterygoideus; *p.q.*, processus quadratus; *prq.*, paraquadratus; *ptg.*, pterygoid; *qmax.*, quadratomaxillary. Other abbreviations as in previous figures.

processus articularis processûs quadrati, which gradually extends above and below the quadrato-maxillary, until the condition sketched in Text-fig. 9 *b* is reached. The bone is now sunk into the processus articularis, so that it is no longer distinguishable from mere enchondral ossification of the latter. The pterygoid has not undergone any important histological changes, but the elongation of the processus pterygoideus proves that the processus basalis is about to be effected. The crista parotica has already appeared, and has acquired relations with the extremely short paraquadrato, which preceded it in sections, strikingly reminiscent of those described above for the quadrato-maxillary and the processus quadratus. The crista is ossified enchondrally as well as perichondrally, and the bony material so formed is seen to effect a synostosis with the paraquadrato. Moreover, that part of the paraquadrato adjacent to this synostosis has lost its underlying connective tissue-layer and simulates perichondrial ossification of the outer face of the crista. The identity of the paraquadrato is however quite apparent in its ventral portion, where the connective tissue is preserved. Upon the establishment of the processus oticus (Text-fig. 9 *c*) the ossification of the crista parotica is separated from the paraquadrato, which then appears in its true light as a membrane bone of the outer surface of the quadrato cartilage. But Text-fig. 9 *c* shows, furthermore, that the pterygoid has also acquired close relationship with the invested processus ascendens in a similar manner to that described for the quadrato-maxillary and paraquadrato, although in this case also it disengages itself from the processus ascendens posteriorly. Whereas, therefore, one bone only, the quadrato-maxillary, invades the cartilage in the frog, the pterygoid and paraquadrato do so too in the case of *Phrynomerus*.

The otic capsule and associated cartilages.

The ossifications in the region of the otic capsule have been discussed above in connexion with the prootic and exoccipital. It remains to give some account of the sound-conducting apparatus, which in its maximal development in *Anura* consists of

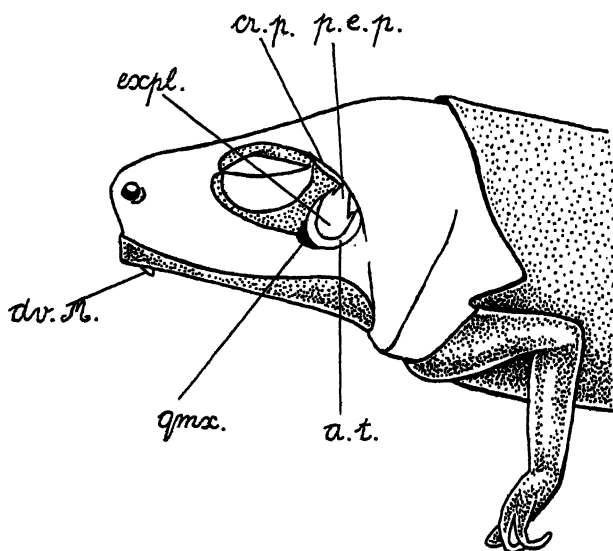
(a) a plectrum, showing three regions : (a, I) a cartilaginous pars externa embedded in the tympanic membrane and stretching across the middle ear ; in *Rana* it is joined to the crista parotica by a pars ascendens plectri. (a, II) The pars media, which ossifies and lies below the crista, is in some cases double. The pars interna (a, III) is a cartilage bar articulating with (b), the operculum lying in the fenestra ovalis. The tympanic membrane, when present, is supported by an iris-like annulus tympanicus, and is in some cases the seat of a disc-like cartilage in continuity with the pars externa plectri. This cartilage has sometimes been called an extra-stapedial (von Bonde, 1919), but the name signifies nothing, as the whole plectral apparatus is extra-stapedial in situation, if we assume, as do many morphologists these days, that Gaupp's plectrum and operculum (*Anura*) are homologous with the columella and stapes in *Sauropsida*. If we accept this interpretation, perfectly justifiable on Abel's (1929) new definition of homology, the cartilage in question would be the homologue of the Reptilian extra-columellare. If not, the cartilage may be preliminarily called with great safety an 'extraplectral'.

It is necessary at this stage to arrive at some idea of the mysterious phrase 'tympanum hidden' (*Tympanum versteckt*), so frequently employed by systematists. I have consulted our leading South African systematist, Mr. Hewitt of Grahamstown, on the subject, and he confirms my interpretation : that by the above phrase is meant the non-differentiation of the general external ectoderm layer of the tympanic membrane, which in reality consists of three layers. A 'hidden tympanum' is in any case a dangerous cliché, for it is never anything but hidden, whether the superficial ectoderm is thin and transparent or thick and undifferentiated. In *Phrynomerus* the region of the annulus tympanicus and its associated membranes lies posterior to the eye and in close proximity to it, and can be clearly seen upon removal of the skin. The otic capsule lies some distance behind the annulus, as already explained above.

The following description of the annulus and the more external parts of the auditory ossicles is based not only on microtomed

series, but also on surface investigation with a stereoscopic microscope. It appears that the annulus is an incomplete ring, the discontinuity being posteriorly situated. Text-fig. 10 is a sketch of the side of the head, showing the sickle-shaped annulus in position, and its relations to the cartilage mass it surrounds.

TEXT-FIG. 10.



Sketch of the left side of the head with the skin removed. *cr.p.*, crista parotica; *dv.M.*, diverticulum of Meckel's cartilage; *p.e.p.*, pars externa plectri. Other abbreviations as in previous figures.

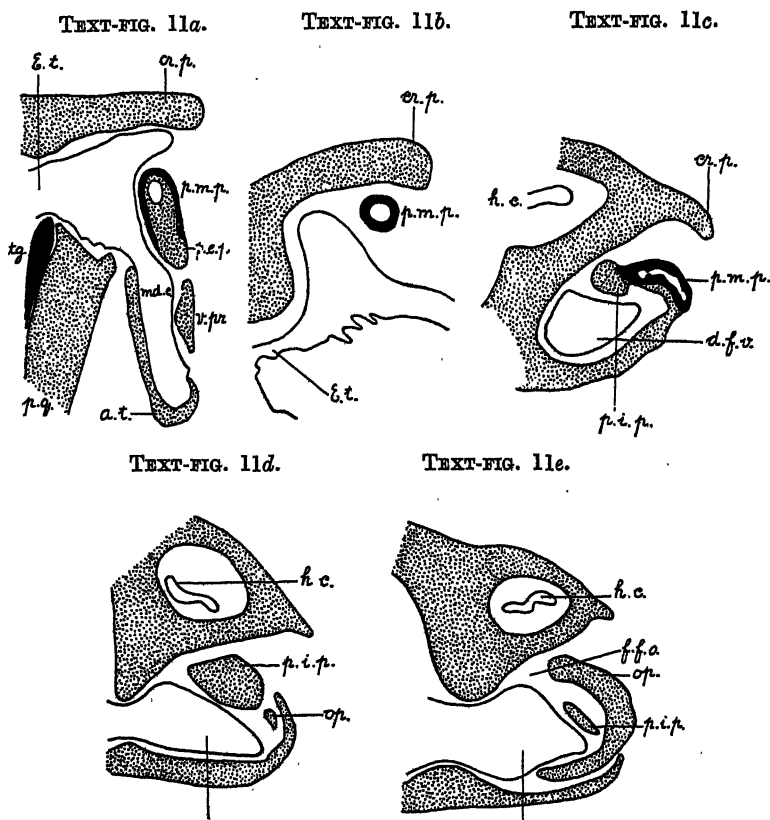
The latter consists of a body, which may be interpreted as representing the pars externa+extrapleural, and a short, dorsal, posteriorly directed cartilage which possibly represents the vestige of the pars ascendens. In section the various cartilages appear as follows: In Text-fig. 9 *a* the annulus and the extrapleural are sectioned and the former is proved to be of the same trough-like type as in the frog. In Text-fig. 9 *b* the dorsal limb of the sickle has disappeared, and the vestige of the processus ascendens plectri is seen above the extrapleural in

Text-fig. 9 *c*. The extrapleural is rapidly attenuated and in Text-fig. 9 *a* it is perichondrally ossified and possesses a dorsal marrow cavity. The ossified portion marks the anterior limits of the pars media. The additional cartilage present in the figure represents a ventral process of the extrapleural. Text-fig. 11 *b* represents a section in the region of the opening of the Eustachian tube; the pars media lies in the connective tissue underneath the crista parotica, to which it is much more closely approximated than in the frog. It is a roundish bone with a central marrow cavity. Text-fig. 11 *c* marks the appearance of the pars interna, as cartilage medial and ventral to the pars media and in cartilaginous continuity with the otic capsule. Text-fig. 11 *d* marks the liberation of the pars interna and the appearance of the operculum, which is seen in the following sections to lie between the pars interna and the otic capsule's ventral ridge. In Text-fig. 11 *e* the pars interna is about to disappear from section and the operculum has the form of a half-moon, lying external to the foramen ovale, in a sort of vestibule termed the fossa fenestrae ovalis, in which is located the ductus fenestrae vestibuli, a diverticulum of the perilymphatic space. The fossa is particularly deep and voluminous in *Phrynomerus*. The operculum is posteriorly prolonged beyond the foramen ovale, its transverse section in this region appearing rounded. It will therefore be seen that the auditory apparatus in *Phrynomerus* is very similar to that of the frog; the main differences will be enumerated in the general résumé.

The lower jaw.

The *Anura* are remarkable for the great reduction of the membrane bones of the lower jaw, the angular (= goniale) and the dentary being the only ones represented. One cartilage bone develops; the mentomandibular or mentomeckelian, in the form of a perichondrally ossified sheath of the cartilage of Meckel, lateral to the mental symphysis. The contained cartilage pith of the mentomandibular shows incipient enchondral ossification in *Rana* (and *Arthroleptella*), but the marrow

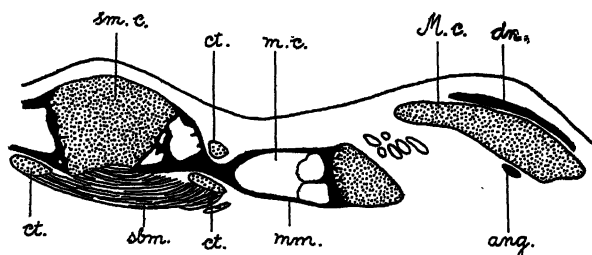
cavity does not entirely oust the remains of Meckel's cartilage. In *Phrynomerus*, however, the latter is the case. It is of



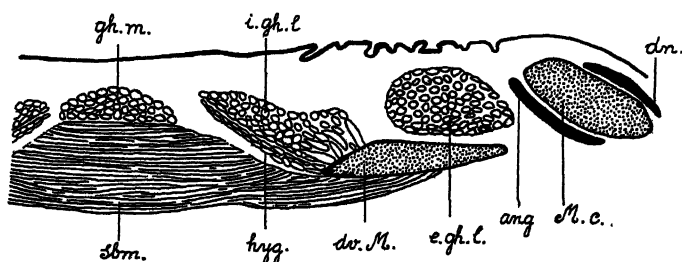
Consecutive transverse sections through the otic region. *d.f.v.*, ductus fenestrae vestibuli; *E.t.*, Eustachian tube; *f.f.o.*, fossa fenestrae ovalis; *h.c.*, horizontal canal; *op.*, operculum; *p.i.p.*, pars interna plectri; *p.m.p.*, pars media plectri; *v.pr.*, ventral process of the extrapleural. Other abbreviations as in previous figures.

great interest, that the medial epiphysis of the mento-mandibular is dorsally and ventrally overlain by cartilage and has its own marrow cavity (Text-fig. 12 a). Upon the disappearance of the

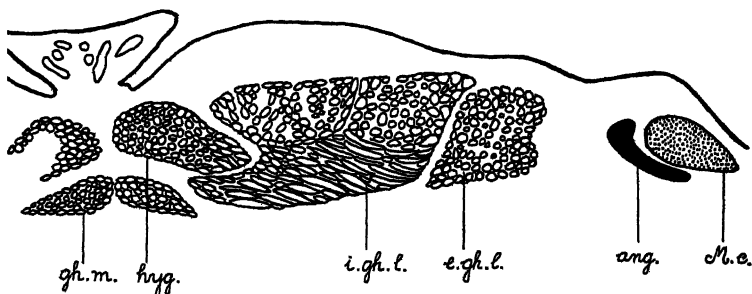
TEXT-FIG. 12a.



TEXT-FIG. 12b.



TEXT-FIG. 12c.



Consecutive transverse sections through the muscles and skeletal structures of the anterior gular region. *ct.*, external cartilaginous ring encasing the mento-mandibular; *e.gh.l.*, external division of the geniohyoideus lateralis; *gh.m.*, geniohyoideus medialis; *hyg.*, hyoglossus; *i.gh.l.*, internal division of the geniohyoideus lateralis; *mm.*, mento-mandibular; *sm.c.*, symphyseal cartilage; *sbm.*, submental. Other abbreviations as in previous figures.

mento-mandibular from sections, its lateral epiphysis persists, and a most extraordinary arrangement results, as this cartilage, weakly ossified on its anteromedial surface, may be traced backwards as a bar running parallel to the lower jaw (Text-fig. 12 b). This peculiarity is to my knowledge perfectly unique among living vertebrates, and an adaptation of doubtful purpose. The musculature of the symphyseal and throat region is considerably modified; the *musculus submentalis* is enlarged and attached to the posterior diverticula of Meckel's cartilage described above. The *mm. geniohyoidei* are now usually treated as two distinct muscles (Bigalke, 1926); the *m. geniohyoideus medialis* and the *m. geniohyoideus lateralis*. The two muscles remain separate as in *Bufo* (Bigalke, 1926), whereas in *Rana* they fuse towards their middle. The *m. geniohyoideus medialis* lies dorsal to the *m. submentalis* as in the frog, but instead of being situated ventral to the *m. hyoglossus* and the *m. geniohyoideus lateralis*, it lies medial to both (Text-fig. 12 b). This arrangement is unquestionably due to the enlargement of the *m. submentalis* and the consequent separation of the two *mm. hyoglossi*. When the latter first appear, they are closely approximated to the medial surface of the medial portion of the *m. geniohyoideus lateralis* (Text-fig. 12 b), and like this muscle, acquire attachment to the Meckelian diverticulum, so that its fibres run transversely. When the Meckelian diverticulum disappears, approximately in the region of the anterior margin of the choanal opening, the *m. hyoglossus* shifts dorsally, to lie between the tongue and the *m. geniohyoideus medialis* (Text-fig. 12 c). The *m. geniohyoideus lateralis* is a large muscle, consisting of two divisions as in *Bufo* and running in close proximity to the lower jaw.

It therefore appears that the Meckelian diverticulum owes its presence to the necessity of a strong attachment for the *m. submentalis*, *m. hyoglossus*, and the medial portion of the *m. geniohyoideus lateralis*, the *m. geniohyoideus medialis* being comparatively small. The most remarkable feature of the musculature of the symphyseal region is, however, the enlargement of the otherwise small *m. submentalis* whose function is

described by Gaupp (1896) in 'Die Anatomie des Frosches', where the closing of the narial apertures is attributed to its contraction. But it is not improbable that an enlarged submentalis may be associated with the loss of teeth as well. Gaupp (1896) describes as the function of the geniohyoidei that of an opener of the mouth and of the narial aperture, so that it is quite comprehensible that enlargement of the geniohyoideus must necessarily entail enlargement of the submentalis. Why the apparatus for opening and closing of the nostrils should be so strongly developed in *Phrynomerus* is not easily explained, since very little is known of the habits of the animal. But it may possibly be associated with the prolonged hibernation of the frog in tree-trunks, &c., during which period there is a diminution, if not actual cessation of respiratory movements.

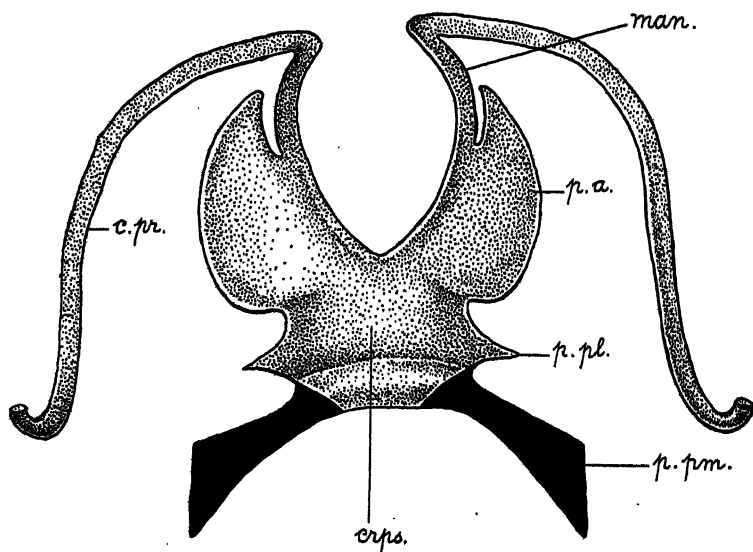
The rest of the lower jaw offers few features of interest; the dentary and angular (= goniale = dermarticulare) are both toothless and conform to the type met with in *Rana*.

The Hyoid Apparatus.

This was dissected out and drawn with the aid of the Günther-Metz apparatus; Text-fig. 18 presents a sketch made from the ventral aspect. The cornua principalia are slender cartilage rods fused synchondrotically with the otic capsule, although the point of fusion can be clearly seen in transverse sections through the otic region. There are no processûs anteriores, so that the manubrium passes into the cornu beyond the sharp angle indicated in the figure. The two angles are joined by tough connective tissue, which however shows no signs of chondrification. The processûs alares are very large blade-like structures, not separated from the corpus by a neck as in *Rana*. The processûs postero-laterales are sharp conical structures transversely situated, not directed backwards as in *Rana*. Owing to the enormous heart-shaped intermanubrial bay, which is continued into the corpus, the latter is much smaller than in *Rana* and is predominantly transversely developed; as a result of this peculiarity, the ossified processûs thyreoidei do not touch each other medially, but are separated by the posterior

boundary of the corpus, which in this region consists of a thick cartilaginous ridge, probably developed in response to the need of extra firmness in the thyroid region of the hyoid apparatus.

TEXT-FIG. 13.

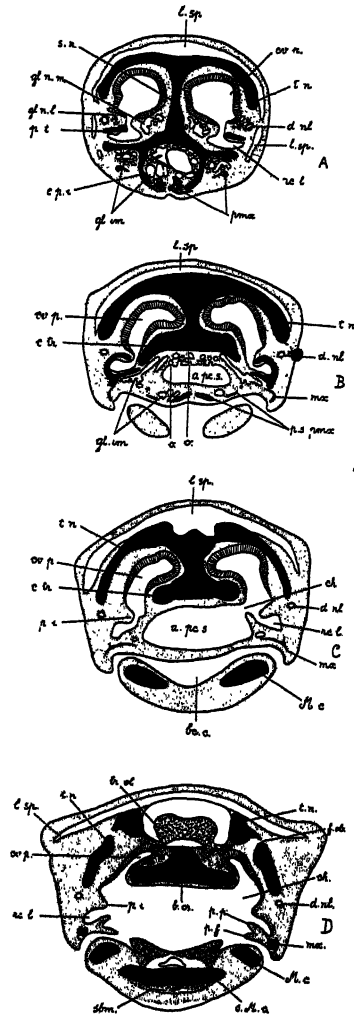


The hyoid apparatus. *c.pr.*, cornu principale (= hyale); *crps.*, corpus; *man.*, manubrium; *p.a.*, processus alaris; *p.pl.*, processus postero-lateralis; *p.p.m.*, processus postero-medialis (thyroid).

The metamorphosed toadlet of *Phrynomerus*.

Mr. J. H. Power of Kimberley, who collected developmental material of *Phrynomerus* at Lobatsi, very kindly supplied the writer with a complete set, from which the young toadlet was microtomed. A very beautiful series of sections was obtained, a study of which has helped towards the elucidation of the peculiar buccal sacs described above. In a series of transverse sections through the head, the glandula intermaxillaris is at first the most conspicuous object (see Text-fig. 14 a). It is bounded dorsally by the solum nasi, ventrally by the two pairs of palatal premaxillary squames, laterally by the

TEXT-FIG. 14.



Consecutive transverse sections through the narial region of a fully metamorphosed toadlet. *b.cr.*, basis cranii; *ch.*, choana; *c.p.i.*, cartilago prae-nasalis inferior; *c.tr.*, cornu trabeculae; *a.n.l.*, ductus naso-lacimalis; *f.ob.*, foramen orbito-nasale; *l.sp.*, lymph space; *o.*, openings of the glandula intermaxillaris into the anlage of the prechoanal sacs (*p.p.c.s.*); *p.i.*, plica isthmi; *p.m.x.*, premaxilla; *p.s.p.m.x.*, palatal aquames of the premaxilla; *rc.l.*, recessus lateralis; *s.M.c.*, symphysial portion of Meckel's cartilage. Other abbreviations as in previous figures.

crista subnasalis, and consists of tubules with remarkably wide lumens. The centre of the intermaxillary gland-mass is next occupied by a large central cavity (Text-fig. 14 *b*) on whose dorsal surface the glandular tubules have numerous openings. The central cavity referred to above is in reality the unpaired anlage of the two prechoanal sacs described in the adult, and is ontogenetically to be considered as a vestibule into which the intermaxillary gland opens. The anatomical details of the gland are described by Krause (1923, p. 457) for *Rana*, in which the openings (20-25) are located half-way between the pulvinar subrostrale and the vomer. If we imagine this region of the buccal cavity to undergo an evagination, the vestibular unpaired anlage of the prechoanal sacs of *Phrynomerus* becomes easily intelligible. The choana of the larval, or at any rate, post-metamorphic *Phrynomerus* also opens into the vestibule, which is therefore in communication with the narial cavity and with the buccal cavity as is the organ of Jacobson in Amniotes (see Text-fig. 14 *c*). The point of communication of the vestibule with the buccal cavity is seen in Text-fig. 14 *d*; the recessus lateralis in *Phrynomerus* has a bifurcated ventral plical boundary, whereas in *Rana* it is single. The difference is however unreal, for when transverse sections of the choanal region of *Rana* and *Phrynomerus* are compared, it becomes clear that the dorsal ridge in *Phrynomerus* is the plica palatalis of *Rana* and the ventral the palatal ridge (=Gaumenfalte of Krause = Gaumenleiste of Gaupp). The correctness of this homology becomes patent when Text-fig. 14 is compared with Text-fig. 14 *d*. The palatal fold is, therefore, not present in *Phrynomerus* prechoanally, since it is fused with its fellow on the opposite side to cover over the unpaired anlage of the prechoanal sacs.

The rest of the anatomy of the head region of the toadlet is not of such great interest, but will be briefly reviewed. The plica obliqua is beginning to develop, but is relatively smaller than in the adult. Of the two 'Wülste', only the larger, taking the place of the alary cartilage, is present. The recessus saciformis is present. The recessus medialis, supposed to represent

the organ of Jacobson in *Anura*, is in close proximity to the glandula intermaxillaris, a fact not devoid of interest, since the analage of the prechoanal sacs is therefore more closely approximated to what might be termed the nasal portion of Jacobson's organ.

The membrane bones of the facial region of the skull are in the process of developing as densifications in the cutis, but the vomer, palatine, and septomaxillary are absent. The maxilla and premaxilla show no traces of dental vestiges.

The fronto-parietals are beginning to develop and show the same relation to the chondrocranium as in the adult. The transverse taenia is absent, but the median is well developed. The parasphenoid is firmly established as a median ventral membrane bone.

The muscles of the gular region have already acquired their adult arrangement and the diverticula of Meckel's cartilage are established. The mento-mandibular is absent, as no cartilage bone whatsoever is as yet present in the skull, but the dentary and articular are beginning to form.

Contrary to expectation, the ear is as yet in a very primitive condition: the annulus tympanicus is entirely absent, and the Eustachian tubes are feebly indicated as shallow evaginations of the buccal cavity, running in a dorso-lateral direction between the pars quadrata palatoquadrati and the point of fusion of the hyale with the otic capsule. The remarkable point about the processus basalis is its apparently autochthonous origin, which was also remarked upon by Gaupp. In *Phrynomerus*, too, the cartilage of the processus basalis is histologically quite distinct from that of the pars quadrata or of the otic capsule, inasmuch as it is more procartilaginous, has many more cartilage cells lying in small cartilage cavities ('Knorpelhöhlen' of German authors) and separated by a comparatively small amount of matrix. Of much morphological interest is the fact that the hyale is not fused to the otic capsule proper, but to the processus basalis, so that the first visceral arch is in cartilaginous continuity with the hyale. It is, however, just possible that the processus basalis is a hyoid derivative, in which case also it

would represent a communication between the first two visceral arches in *Phrynomerus*. The plectrum, according to Gaupp, develops independently of the lower margin of the foramen ovale, but subsequently effects a fusion with it. The present author does not consider the development of the plectrum from the lower margin as being conclusively disposed of. In any case the plectrum is in the young *Phrynomerus* a cartilaginous rod only partially separated from the lower margin and not differentiated into the three 'partes'. The operculum has the usual bowl-like shape and possesses an external ridge to which a rudimentary muscle is attached. Muscle and ridge are totally absent from the adult. According to Versluys (1924, deel II, p. 376) the operculum is an auditory cartilage developed in response to the needs of terrestrial life; in that case *Phrynomerus* must be on its way to becoming less terrestrial or more aquatic, since a more complicated opercular apparatus is recapitulated during the ontogeny. It is also very remarkable that *Cacosternum*, which I would call an aquatic toad, has a very large operculum with its attendant *musculus opercularis*. The development of the operculum is still disputed: Gaupp described as its anlage connective tissue filling the posterior division of the fenestra ovalis, but Versluys, quoting recent work of Kingsbury and Reed, accepts its origin from the wall of the capsule. In the young *Phrynomerus* the operculum is free from the lower lip of the foramen ovale, but is pressed lightly against the upper. There is, however, no cartilaginous continuity, the articulation being effected by the perichondria only. Finally, it should be stated that a *pars ascendens plectri* is absent in the young as well as in the adult *Phrynomerus*. In fact this commissure between the plectrum and the *crista parotica* is absent in all South African *Anura* examined by me, although most text-books of comparative anatomy, under the influence of what one may be forgiven for terming the *Rana* complex, assume a universal presence of the process in *Anura*.

The paraquadrata is already developed as a membrane bone on the outer face of the *pars quadrata palatoquadrati*; the

pterygoid is present on its inner surface, but does not invest the processus basalis. No trace of the quadratomaxillary is to be found yet.

The hyoid apparatus still lacks the processûs postero-laterales, and the processûs alares are not clearly demarcated from the corpus. The thyreoids (processûs postero-mediales) are unossified.

RÉSUMÉ.

It is proposed to follow up this paper by one on *Cacosternum*, in which the mutual affinities of the two South African genera lacking a procoracoid will be discussed. In the meantime a short résumé of those points in which *Phrynomerus* differs from *Rana* with respect to its cranial characters may be useful.

1. The plica obliqua is knob-like and suspended from the cartilage obliqua, not from the tectum nasi.

2. The recessus sacciformis is absent in the adult, but recapitulated in the young.

3. There are remarkable prechoanal sacs in the adult, developed from an unpaired anlage into which the choanae and the glandula intermaxillaris open. The apparatus possibly represents remains of the buccal division of Jacobson's organ, not previously described for anamniote tetrapods.

4. The eminentia olfactoria is very high and supported by a cartilaginous axis.

5. The bones of the secondary upper jaw are edentulous in the adult and the young; the same applies to the vomer and dentary.

6. The premaxilla and maxilla are separated in the palatal region by a retrally flexed process of the crista subnasalis.

7. The vomer surrounds the anterior and medial margins of the choana and therefore has a sickle-shaped form. It is fused with the palatine to form a vomero-palatine.

8. The posterior bay in the nasal is deep and the bone does not articulate with the maxilla.

9. The 'os en ceinture' consists of two entities separated by permanently cartilaginous material dorsal to the parasphenoid.

10. The optic foramen is not surrounded by cartilage, but is bounded anteriorly by the 'os en ceinture' and posteriorly by the prootic.

11. The prootic does not send off diverticula dorsal and ventral to the crista parotica.

12. The taenia medialis is absent from the adult, but present in the young.

13. The transverse taenia is absent in both, so that a fenestra parieto-dorsalis results.

14. The fronto-parietals, in the adult as well as in the young, do not meet in the middle line, so that a large parieto-frontal foramen results.

15. The pterygoid and paraquadrate effect an invasion of the invested cartilage similar to the invasion of the quadrate cartilage by the quadrato-maxillary.

16. The annulus tympanicus is not a complete 'annulus', but sickle-shaped.

17. The pars externa plectri is enlarged to form an extra-plectral.

18. The pars ascendens plectri is absent in the young, probably also in the adult, or possibly reduced to a mere vestige.

19. The elongated crista parotica and processus oticus palato-quadrati are responsible for the separation of the permanent suspensorium from the otic capsule.

20. The general ectoderm is not specially modified over the tympanal portion of the middle ear.

21. The lower jaw has a pair of backwardly directed diverticula of Meckel's cartilage, the presence of which is associated with considerable modification of the musculature of the gular region.

22. The hyoid apparatus lacks a processus anterior in the adult, although they are feebly indicated in the young. The corpus is small and the alary processes large and blade-like.

23. The hyale fuses with the processus basalis of the young.

LIST OF LITERATURE CITED.

- Abel, O. (1929).—‘Stammesgeschichte und Paläobiologie.’ Fischer, Jena.
- Bianconi, J. J. (1850).—‘Specimina zoologica mosambicana.’ Bononiae.
- Bigalke, R. (1926).—‘Zur Myologie der Erdkröte, Dissertation.’ Berlin.
- Boulenger, G. A. (1882).—‘Catalogue of the Batrachia salientia sive Ecaudata in the collection of the British Museum.’
- (1906-9).—“A revised list of the South African Reptiles and Batrachians”, ‘An. S. African Mus.’, vol. v, p. 455.
- Broman, I. (1919).—“Das Organonvomero-nasale Jacobsoni—ein Wassergeruchsorgan!”, ‘Anat. Hefte’, Bd. 58, S. 143.
- Broom, R. (1902).—“On the Mammalian and reptilian vomerine bones”, ‘Proc. Lin. Soc. N.S.W.’, vol. 28, part 4.
- (1915).—“Origin of Mammals”, ‘Phil. Trans. Roy. Soc., London’, vol. 206 B.
- de Villiers, C. G. S. (1929).—“The comparative anatomy of the breast-shoulder apparatus of the three aglossal Anuran genera: *Xenopus*, *Pipa*, and *Hymenochirus*”, ‘An. Tvl. Mus.’, vol. xiii, p. 37.
- (1929).—“On the development of a Jonkershoek species of *Arthroleptella*”, ‘S. African Journ. of Science’, vol. xxvi.
- Gaupp, E. (1892).—“Beiträge zur Morphologie des Schädels. I. Primordial-Cranium und Kieferbogen von *Rana fusca*”, ‘Morphol. Arb.’, Bd. II, S. 275.
- (1894).—“Beiträge zur Morphologie des Schädels. III. Zur vergleichenden Anatomie der Schläfengegend am knöchernen Wirbeltierschädel”, *ibid.*, Bd. IV, S. 77.
- Gaupp, E., Wiedersheim, R. und Ecker, A. (1896).—‘Anatomie des Frosches’, Bd. I. Vieweg, Braunschweig.
- (1904).—Bd. III.
- Gaupp, E. (1906).—“Kopfskelett” in O. Hertwig’s ‘Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere’, Bd. III, Teil 2 und 3, S. 573.
- Günther, A. (1858).—‘Catalogue of the Batrachia salientia in the collection of the British Museum.’
- Hewitt, J. (1911).—“Records of South African Lacertilia and Amphibia”, ‘An. Tvl. Mus.’, vol. iii, p. 42.
- (1919).—“*Anhydrophryne rattrayi*, a remarkable new frog from Cape Colony”, ‘Rec. Alb. Mus.’, vol. iii, p. 182.
- Kothe, K. (1910).—“Entwicklungsgeschichtliche Untersuchungen über das Zungenbein und die Ohrknöchelchen der Anuren”, ‘Archiv f. Natges.’, Bd. 76.
- Krause, R. (1923).—‘Mikroskopische Anatomie der Wirbeltiere in Einzeldarstellungen. III. Amphibien.’ De Gruyter, Berlin.

- Loveridge, A. (1925).—"Notes on East African Batrachians, collected 1920-3, with description of four new species", 'Proc. Zool. Soc.', p. 763.
- Mayer, P. (1920).—"Zoomikrotechnik." Bornträger, Berlin.
- Nieden, F. (1926).—"Anura II, Engyostomatidae, Das Tierreich," 49ste Liefg.
- Noble, G. K. (1922).—"The phylogeny of the Salientia. I. The osteology and the thigh musculature: their bearing on classification and phylogeny", 'Bull. Am. Mus. Nat. Hist.', vol. xlv, p. 1.
- (1924).—"Amphibia", in "Contributions to the Herpetology of the Belgian Congo", *ibid.*, vol. xlix, p. 147.
- (1926).—"An analysis of the remarkable cases of distribution among the Amphibia, with descriptions of new genera", 'Am. Mus. Nov.', No. 212.
- (1926 a).—"A synopsis of the Brevicipitid Toads of Madagascar", *ibid.*, No. 232.
- (1926 b).—"The importance of larval characters in the classification of South African salientia", *ibid.*, No. 237.
- (1927).—"The value of life-history data in the study of the evolution of the Amphibia", 'An. N.Y. Ac. Sc.', vol. xxx, p. 31.
- Peters, W. (1867) (quoted in Boulenger, 1882).—"Monatsb. Ak. Wis. Berlin."
- Peters, W. C. H. (1882).—"Naturwissenschaftliche Reise nach Mosambique, Zoologia III: Amphibien", p. 172. Reimer, Berlin.
- Power, J. H. (1926).—"Notes on the habits and life-histories of certain little-known Anura, with descriptions of the tadpoles", 'Trans. Roy. Soc. S. Africa', vol. xiii, part ii, p. 107.
- (1927).—"On the herpetological fauna of the Lobatsi-Linokana area", *ibid.*, vol. xiv, part iv, p. 405.
- Smith, A. (1849).—"Illustrations of the Zoology of South Africa", Reptile volume. Smith, Elder, London.
- Stadtmüller, F. (1929).—"Nachweis eines Basisoccipitale bei einem rezenten Amphibium, Vorweisungen zur dritten wissenschaftlichen Sitzung der anatomischen Gesellschaft (Tübingen)", 'Ergänzungsheft zum 67en Band des Anat. Anz.', S. 219.
- von Bonde, C., and Gilchrist, J. D. F. (1919).—"The Platana and the Frog." Cape Times, Cape Town.
- Versluys, J. en anderen (1924).—"Leerboek der vergelijkende ontleedkunde van de Vertebraten", 2 deelen. Oosthoek, Utrecht.
- Wager, V. A. (1926).—"Breeding habits and life-histories of some of the Transvaal Amphibia", 'Trans. Roy. Soc. S. Africa', vol. xiii, part ii, p. 163.
- (1929).—"Breeding habits and life-histories of some of the Transvaal Amphibia, II", *ibid.*, vol. xvii, pt. ii, p. 125.

Werner, F. (1910).—"Reptilia et Amphibia" in 'Zoologische und anthropologische Ergebnisse einer Forschungsreise im westlichen und zentralen Südafrika', Bd. IV, S. 294.

GENERAL KEY TO THE ABBREVIATIONS EMPLOYED AND THE FIGURES IN WHICH THEY ARE FIRST INTRODUCED AND EXPLAINED.

3, *ang.*; 9, *a.t.*; 3, *bc.c.*; 14, *b.cr.*; 8, *bl.v.*; 9, *c.cr.b.*; 14, *ch.*; 1, *c.i.*; 7, *cn.t.*; 14, *c.p.i.*; 13, *c.pr.*; 1, *cr.ob.*; 10, *cr.p.*; 13, *crps.*; 12, *ct.*; 14, *c.tr.*; 3, *cv.i.*; 1, *cv.m.*; 1, *cv.p.*; 8, *diac.*; 8, *dic.*; 7, *d.l.t.*; 11, *d.f.v.*; 3, *dn.*; 14, *d.nl.*; 9, *d.pr.*; 10, *dv.*; 3, *e.b.c.*; 12, *e.gh.l.*; 1, *ec.ves.*; 3, *e.ol.*; 11, *El.t.*; 9, *expl.*; 11, *f.f.o.*; 6, *f.o.*; 14, *f.ob.*; 6, *f.pr.*; 7, *frp.*; 12, *gh.m.*; 3, *gl.im.*; 8, *gln.*; 1, *gln.l.*; 1, *gln.m.*; 11, *h.c.*; 12, *hyg.*; 12, *i.gh.l.*; 1, *inf.*; 2, *i.v.r.s.*; 1, *l.s.*; 14, *l.sp.*; 3, *l.s.v.*; 1, *l.w.o.c.*; 13, *man.*; 7, *m.c.*; 3, *M.c.*; 9, *md.e.*; 8, *mes.*; 12, *mm.*; 8, *ms.*; 7, *m.tr.*; 3, *mx.*; 1, *na.*; 14, *o.*; 6, *o.c.*; 8, *ocl.*; 9, *o.cr.p.*; 6, *o.e.c.*; 11, *op.*; 13, *p.a.*; 7, *par.*; 8, *p.b.*; 3, *pc.s.*; 10, *p.e.p.*; 4, *p.f.*; 14, *p.i.*; 11, *p.i.p.*; 1, *pl.ob.*; 3, *p.m.*; 11, *p.m.p.*; 14, *pmx.*; 4, *p.p.*; 13, *p.pl.*; 13, *p.pm.*; 9, *p.pt.*; 9, *p.q.*; 6, *pr.*; 9, *prq.*; 8, *p.s.*; 14, *p.s.pmx.*; 3, *p.t.*; 9, *ptg.*; 9, *qmx.*; 14, *rc.l.*; 12, *sbm.*; 12, *sm.c.*; 14, *s.M.c.*; 3, *s.n.*; 3, *so.n.*; 1, *spmx.*; 1, *t.n.*; 7, *tr.ol.*; 3, *v.*; 1, *ves.*; 11, *v.pr.*; 2, *v.v.r.s.*; 1, *W.I.*; 2, *W.II.*

C. G. S. de Villiers,
Stellenbosch,
27th Nov. 1929.



The Early Development of the Chondrocranium of the Lizard.

By

G. R. de Beer, M.A., B.Sc., F.L.S., C.M.Z.S.,

Fellow of Merton College, Lecturer of Magdalen College, Jenkinson Lecturer in Embryology, and University Demonstrator in Zoology, Oxford.

With 28 Text-figures.

CONTENTS.

	PAGE
I. INTRODUCTION	707
II. DESCRIPTION OF STAGES	709
EXPLANATION OF LETTERING	710
III. DISCUSSION	730
A. The nasal capsule	731
B. The fenestra ovalis and the basicapsular fenestra	733
C. The planum supraseptale, ala orbitalis, and interorbital septum	734
D. The phylogenetic position of the Lacertilia in the light of the chondrocranium	735
IV. SUMMARY	737
V. LIST OF LITERATURE CITED	773

I. INTRODUCTION.

THE embryonic skull of the lizard was one of the first to be studied by modern critical methods, and now the morphology of the chondrocranium of the Lacertilia may be regarded as well known, thanks to the work of Gaupp on *Lacerta agilis* (31 mm. stage (1900) and 47 mm. stage (1906)), of Rice (1920) on *Eumeces* (various stages), and of Pearson (1921) on *Lygosoma*. However, with the exception of Leydig's (1872) work on *Lacerta* and *Anguis*, Parker's (1879) on *Lacerta*, and Sewertzoff's paper (1900) on *Ascalabotes*, none of which are very detailed, practically no

investigations have been made into the embryology of the lacertilian chondrocranium. The present paper is an attempt to fill this gap, as a result of a study of some two dozen embryos of *Lacerta agilis* of varying stages of development, prepared according to van Wijhe's method (1902), as amended (1922), using victoria blue.

The material was obtained and preserved by Professor E. S. Goodrich in Naples, and I wish here to record my gratitude to him for very kindly turning some of it over to me for this work. The embryos were removed from their shells, and only those which were living and healthy were fixed and used. Unfortunately, it was impossible to determine the respective ages of embryos of the different stages, and it was necessary to have recourse solely to measurements. The shape of the lacertilian embryo is such that the body from the tip of the snout to the tip of the tail is coiled twice on itself, with the result that the so-called 'greatest length', from the prominence of the mid-brain to the root of the tail, is very variable, and depends on the degree of tightness of the coil. For comparative purposes, therefore, measurements of greatest length are of little use, and the investigator is driven to adopting the head-length as his standard of comparison. The various stages figured and described in this paper are enumerated below.

<i>Stage.</i>	<i>Embryo.</i>	<i>Head-length.</i>
		mm.
1	X	2.25
2	A	2.5
3	G	3.5
4	H	4
5	I	4
6	B	4.5
7	C	5
8	D	5.25
9	E	5.25
10	F	5.5

The most advanced stage described in this paper leads on conveniently to the younger of those which Gaupp worked at. Gaupp's model, reproduced in wax by Ziegler, was used as a standard of comparison and as a check to the interpretation

of the drawings which were made directly from the preparations under a camera lucida. In addition, a number of sets of serial sections of *Lacerta* at various stages were used to confirm the reconstructions. It should be remembered that the victoria-blue method of van Wihje is specific for chondrin to the exclusion of procartilage, with the result that embryos prepared by this method may appear to be unduly delayed in their chondrification as compared with reconstructions made from sections of the same stages. In the latter case, it almost always happens that the line of demarcation between cartilage and procartilage is interpreted very liberally for the cartilage. In any case, the differences are slight, and the matter is of little importance.

The work was done in the Department of Zoology and Comparative Anatomy of the Oxford University Museum, in which I enjoyed the unfailing encouragement of Professor Goodrich.

II. DESCRIPTION OF STAGES.

Stage 1 (embryo X, H.L. 2.25 mm., Text-fig. 1).—The first part of the cartilaginous skeleton to chondrify is Meckel's cartilage, and some of the embryos of this stage show no other visible skeleton. In others, however, it is possible to make out a thin film of cartilage on each side of the notochord, beneath the hind-brain, and continuous posteriorly with a small uprising occipital arch on each side. All the roots of the hypoglossal nerve emerge freely in front of the occipital arch of their own side, passing over the parachordal. In such embryos, the chondrification of Meckel's cartilage is obviously more advanced than that of the parachordal and occipital arch. The lizard must therefore be counted among those forms in which the splanchnocranium develops before the neurocranium.

Stage 2 (embryo A, H.L. 2.5 mm., Text-figs. 2 and 3).—The chief difference between this and the previous stage is the fact that the auditory capsule has put in its appearance, as a thin film of cartilage moulded round the lateral surface of the utricle. It must also be noticed that the hindmost of pair roots of the hypoglossal nerve has been enclosed in a foramen by a bar of cartilage which projects sideways and upwards from the para-

chordal to join the occipital arch behind it, on each side. The bar of cartilage in question may be regarded as a preoccipital arch, similar to that which has been demonstrated in *Scyllium* and in *Amblystoma* by Goodrich (1911 and 1918), and in *Lepus* by de Beer and Woodger (in the press). In *Lacerta*

TEXT-FIGS. 2, 3.

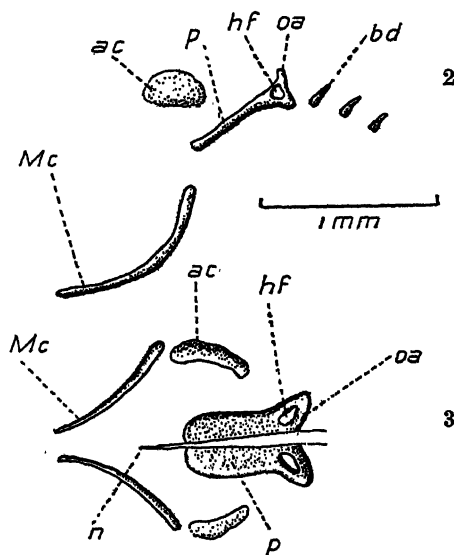


Fig. 2.—Lateral view from left side of the skull.

Fig. 3.—Dorsal view of embryo A, stage 2, H.L. 2.5 mm.

there are eventually (see Text-figs. 7 and 8) three such arches on each side, in front of the occipital arch. Between them these arches will enclose the three roots of the hypoglossal nerves in

processus paroticus; *pr*, processus retroarticularis of Meckel's cartilage; *ps*, planum suprasetale; *pt*, parietotectal cartilage of nasal capsule; *q*, quadrate cartilage; *r*, raphe between lateral and medial nasal processes, leading to aperture of Jacobson's organ; *re*, recessus extraconchalis; *ret*, ramus ethmoidalis of profundus nerve; *rl*, ramus lateralis of ethmoid nerve; *rm*, ramus medialis of ethmoid nerve; *si*, interorbital septum; *sin*, subiculum infundibuli; *sn*, nasal septum; *t*, trabecula cranii; *tc*, trabecula communis; *tl*, true lateral wall of nasal capsule, forming inner wall of cavum conchale; *tm*, taenia marginalis; *tme*, taenia medialis; *ts*, tectum synoticum.

separate foramina. This condition is similar to that which Sewertzoff (1897) observed in *Ascalabotes*. At the stage in question in *Lacerta* (stage 2), the two anterior pairs of roots of the hypoglossal nerve are still free.

Stage 3 (embryo G, H.L. 3.5 mm., Text-figs. 4, 5, and 6).—

TEXT-FIGS. 4-6.

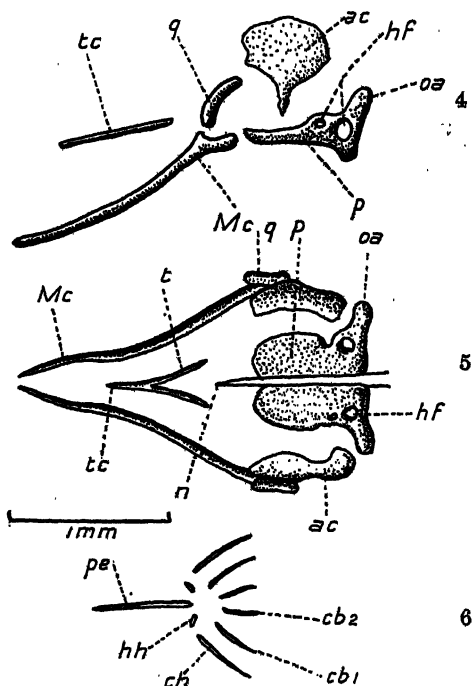


Fig. 4.—Lateral view from left side of the skull.

Fig. 5.—Dorsal view of embryo G, stage 3, H.L. 3.5 mm.

Fig. 6.—Ventral view of hyoid and branchial arches of embryo G.

At this stage, the trabeculae cranii have made their appearance as a pair of bars of cartilage, in front of the parachordals. Posteriorly, the trabeculae diverge from one another, but anteriorly they converge and fuse to form the trabecula communis, which extends forwards for a little way. It has not been possible to find a stage in cartilage at which the trabeculae were not already fused to form a trabecula communis. The

auditory capsule shows a fine process directed downwards, and slightly towards the lateral edge of the parachordal of its own side. In front of this process is the quadrate, which is not, as yet, in contact with either Meckel's cartilage or the auditory capsule. Posteriorly, the enclosure of the hypoglossal root is proceeding, but unequally on the two sides. On the left, the second root is definitely enclosed, while, on the right, it runs through a deep notch. In some embryos at this stage the incipient chondrification of the columella auris may be observed.

The so-called 'hyoid' skeleton at this and the subsequent stages is in an interesting condition. There is a median processus entoglossus (or processus lingualis) corresponding to a basihyal and chondrifying independently. On each side of the posterior end of this basihyal is a pair of separate nodules of cartilage which represent the hypohyals, and lateral to them are the ceratohyals in the form of thin rods extending backwards and outwards. Between the ceratohyals are two more pairs of rods of cartilage, chondrifying independently. These are the first and second ceratobranchials. Whereas at these stages all these cartilages are still separate, they eventually all fuse together, as shown in fig. 386 on p. 771 of Gaupp's (1906) description of a 47 mm. embryo. The hypohyal and ceratohyal of each side then form the cornu hyale or anterior horn of the hyoid. Similarly, the first ceratobranchial gives rise to the cornu branchiale primum and the second ceratobranchial to the cornu branchiale secundum. The latter structure represents a chondrification of the skeletal elements of the fourth visceral arch, and it would seem to be incomplete, for, as will be seen below, another cartilage belonging apparently also to the fourth visceral arch appears at later stages.

Stage 4 (embryo H, H.L. 4 mm., Text-figs. 7 and 8).—The trabecula communis has extended forward between the eyes towards the septum separating the nasal sacs, but posteriorly, the hind ends of the trabeculae cranii are still free from the front of the parachordals. Where the anterior edge of the parachordals touches the notochord there has been no advance, but farther to the side a cartilaginous process is directed for-

ward towards the hind ends of the trabeculae cranii. In this way, the front of the parachordals comes to present a hemispherical concavity from the centre of which the notochord projects. This concavity marks the hind border of the future fenestra basicranialis. The lateral edge of the parachordal shows a little prominence which is directed towards the downward projection

TEXT-FIGS. 7, 8.

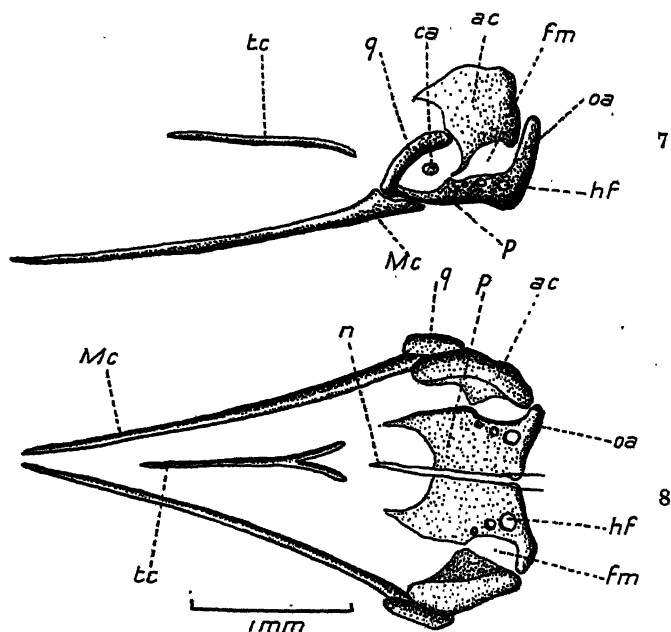


Fig. 7.—Lateral view from the left side of the skull.

Fig. 8.—Dorsal view of embryo H, stage 4, H.L. 4 mm.

from the auditory capsule of its own side, but as far as can be made out is still free from it. Behind this prominence all three roots of the hypoglossal nerve are now enclosed in separate foramina on each side. The occipital arches have extended upwards behind the auditory capsules, and it is now possible to outline a space comprised between the auditory capsule, the lateral edge of the parachordal, and the occipital arch, which space will eventually become the fissura metotica. The hind-

most portions of the parachordal of each side are now extending towards one another beneath the notochord, which will result in the formation of the hypochordal commissure. The chondrification of the auditory capsule is farther advanced, and portions of the roof as well as the septa of the semicircular canals are

TEXT-FIGS. 9, 10.

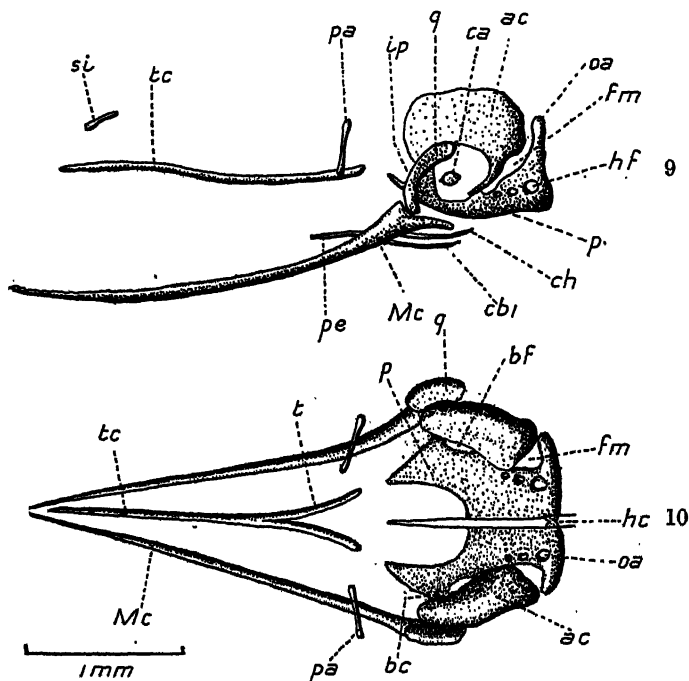


Fig. 9.—Lateral view from the left side of the skull.

Fig. 10.—Dorsal view of embryo I, stage 5, H.L. 4 mm.

present. The walls of the cochlear portion of the auditory capsule are, however, still membranous. Part of the columella auris is now present and can be seen as an independent nodule of cartilage situated behind the quadrate, in the middle of that persistently membranous portion of the side wall of the auditory capsule which will eventually become the fenestra ovalis. This nodule represents the proximal portion of the columella auris,

the so-called otostapes. The quadrate is now articulated with Meckel's cartilage ventrally and with the side wall of the auditory capsule dorsally. No cartilaginous connexion between the quadrate and the columella auris was observed at this or any other stage.

Stage 5 (embryo I, H.L. 4 mm., Text-figs. 9 and 10.)—Two new features have appeared at this stage, viz. the interorbital septum and the processus ascendens. The interorbital septum has begun to chondrify as a little strip of cartilage dorsal to the trabecula communis. Each processus ascendens is a bar of cartilage in a more or less vertical position, in front of the auditory capsules on each side, and quite free from any other cartilage. The most interesting features of this stage concern the relations of the auditory capsule to the parachordal, and here, unfortunately, the results are not as definite as in other regions. It may be stated at once that the investigation of the embryology of the chondrocranium of the lizard by means of the van Wijhe technique has been more difficult than in the case of any other type of vertebrate. The extreme fineness of the strips of cartilage and the difficulties attending the dissection of the preparations prior to mounting have been a handicap to the interpretation of the relations in the more complicated regions. This is especially the case in the region in question, principally owing to the apparent superposition of structures seen in a total preparation. However, by careful comparison between several preparations and reference to serial sections, the following points can be made out. The cochlear portion of the auditory capsule becomes chondrified (in continuity with the rest of the capsule) and acquires a connexion with the parachordal. From the earliest of these stages it seems that the facial nerve is enclosed in its facial foramen, and therefore the connexion between the cochlear part of the auditory capsule and the parachordal must be composed of a prefacial commissure as well as an anterior basicapsular commissure.

Farther back, the process which at earlier stages was described as projecting towards the prominence on the lateral edge of the parachordal now comes into contact with the latter. It is

very important to define this process, and it may be described as the strip of cartilage which forms the lateral and anterior borders of the foramen perilymphaticum of the definitive auditory capsule.

The result of these relations is that between the auditory capsule and its process described in the previous paragraph, the lateral edge of the parachordal, and the anterior basicapsular commissure, there is a gap which at this stage is free from cartilage. Into the upper portion of this gap the proximal end of the columella auris projects, and so this portion of the gap may be regarded as the fenestra ovalis. The lower portion of the gap, however, owes its existence solely to the delay in chondrification of the floor of the cochlear portion of the auditory capsule. While the van Wijhe preparations show a clear space in this region, sections reveal the presence of procartilage in an early stage of histological differentiation, and this procartilaginous floor of the auditory capsule is in contact with the lateral edge of the parachordal. These relations are of importance in view of the question of the relation of the fenestra ovalis of the auditory capsule to the so-called basicapsular fenestra of other forms. At the present stage in the development of the lizard, the fenestra ovalis is present, and it is continuous with a space which may be called the basicapsular fenestra and which represents merely the as yet unchondrified floor of the auditory capsule.

Anteriorly, the parachordals are still free from the trabeculae, while posteriorly they have met beneath the notochord to form a hypochordal commissure.

Stage 6 (embryo B, H.L. 4.5 mm., Text-figs. 11 and 12).—The hind ends of the trabeculae cranii have now established connexion with the anterior projections of the parachordals, with the result that a large pear-shaped gap is enclosed in the floor of the skull. This gap represents the conjoined fenestra basicranialis and fenestra hypophyseos of later stages, which have not yet become separated from one another. Opposite the base of each processus ascendens is a small lateral projection from the hindmost region of each trabecula cranii, forming the

basitrabecular process. Farther forward, the interorbital septum is now more extensive and has become connected with the trabecula communis below, and with a new structure, the

TEXT-FIGS. 11, 12.

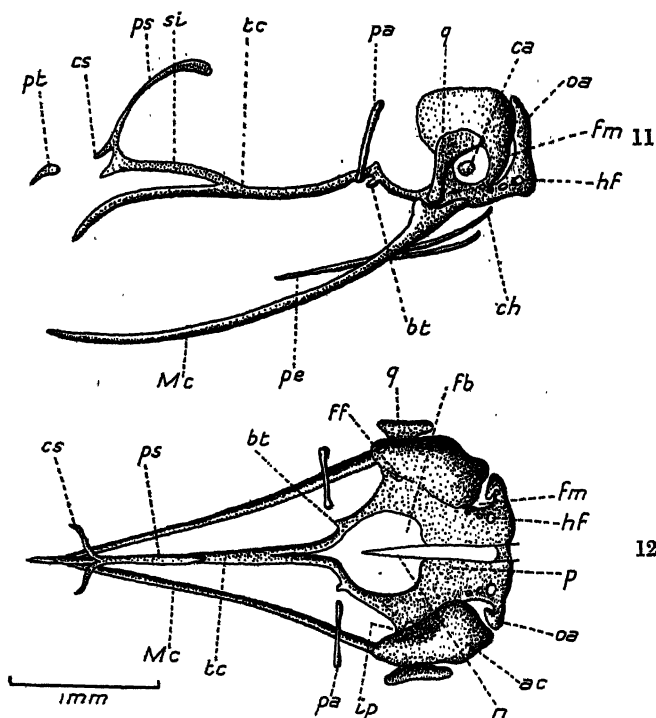


Fig. 11.—Lateral view from the left side of the skull.

Fig. 12.—Dorsal view of embryo B, stage 6, H.L. 4.5 mm.

planum, suprasetale above. The latter is really a paired structure of which the two members have met in the middle line dorsal to the interorbital septum and beneath the brain. Anteriorly the planum suprasetale is continuous with a pair of processes which project to each side: the rudiments of the sphenethmoid commissures. In front of this again, the roof of the nasal capsule is beginning to chondrify in the form of the parietotectal cartilage. The trabecula communis now extends

between the paired nasal sacs and gives rise in this region to the nasal septum.

As regards the auditory capsule, the floor has now become cartilaginous, with the result that the fenestra ovalis has a

TEXT-FIGS. 13, 14.

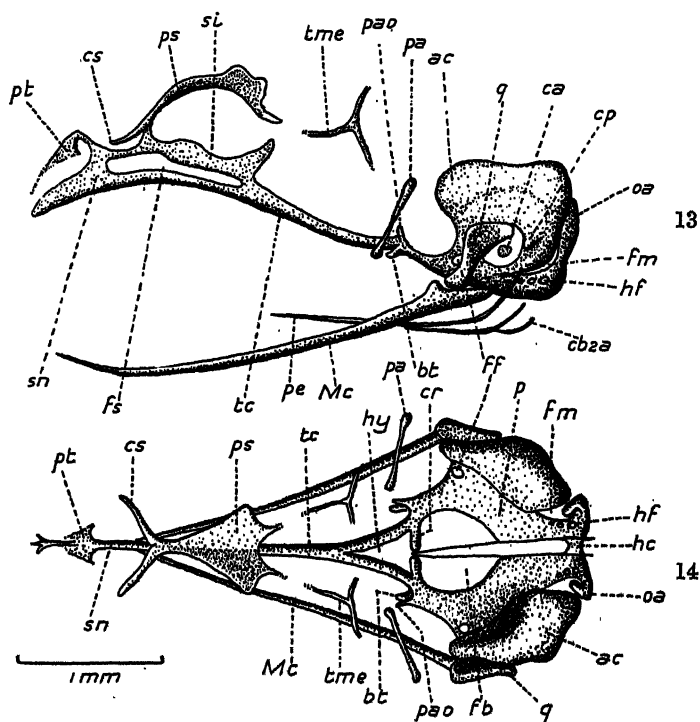


Fig. 13.—Lateral view from the left side of the skull.

Fig. 14.—Dorsal view of embryo C, stage 7, H.L. 5 mm.

median border. The walls and roof of the capsule are now well formed, and the quadrate abuts against a prominence formed by the lateral semicircular canal.

It is interesting to note that at this stage the trabeculae make a fairly sharp angle with the parachordals.

Stage 7 (embryo C, H.L. 5 mm., Text-figs. 13 and 14).—The nasal and interorbital septa are now more extensive,

although unchondrified gaps remain in the form of septal foramina. Anteriorly, the parietotectal cartilages, which form the roof and part of the side of the nasal capsule, grow out of the nasal septum. The sphenethmoid commissures still end freely in front, but the planum supraseptale has enlarged to form a plate immediately underlying the end-brain. In the orbito-temporal region, the rudiments of the side wall of the skull are appearing in the form of a few struts, in which part of the taenia medialis (taenia parietalis media) can be recognized. The basitrabecular processes are further developed and immediately above them is the small rudiment of the pila antotica.

At the place where the trabeculae and parachordals met, there is from the point of junction on each side a process projecting inwards towards its fellow of the opposite side, and tending to divide the original large pear-shaped gap in the floor of the skull into an anterior fenestra hypophyseos and a posterior fenestra basicranialis. These processes form the rudiment of the crista sellaris, and its paired origin is unexpected. As far as can be made out, the space between the two halves of the crista sellaris in the middle line is occupied by the anterior end of the notochord. The angle between the planes of the trabeculae and of the parachordals, which was marked at the previous stage, has now been smoothened out to a considerable extent.

The side wall of the auditory capsule bears a projection which juts out from the prominence for the lateral semicircular canal, behind the head of the quadrate. This is the crista parotica, to which, as Gaupp (1900 and 1906) showed, a structure which was in blastematous continuity with the columella auris and known as the processus paroticus, becomes attached. This processus paroticus eventually chondrifies, as will be seen in stage 9.

As regards the splanchnocranium, the posterior or dorsal portion of the skeleton of the fourth visceral arch has now chondrified, as a pair of rods lying between the hind ends of the first ceratobranchials.

Stage 8 (embryo D, H.L. 5.25 mm., Text-figs. 15 and 16).—

The chief advance which this stage shows concerns the side wall of the skull in the orbitotemporal region. The rudiment of the taenia medialis of the previous stage is now connected with

TEXT-FIGS. 15, 16.

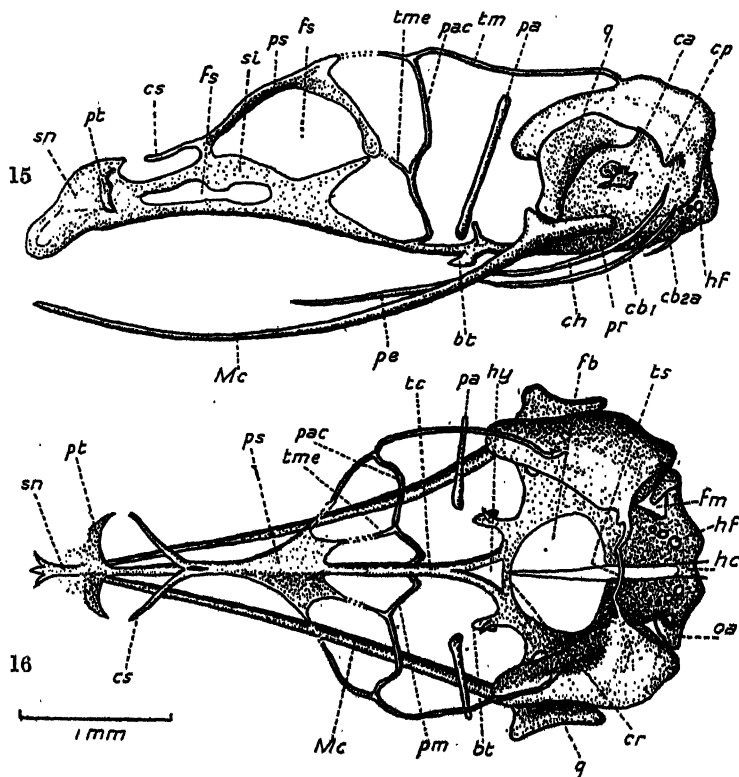


Fig. 15.—Lateral view from the left side of the skull.

Fig. 16.—Dorsal view of embryo D, stage 8, H.L. 5.25 mm.

the trabecula communis by means of the pila metoptica, and dorsally it is continuous by means of the pila accessoria with the taenia marginalis which extends forward as a slender strip of cartilage from the roof of the auditory capsule. Neither the taenia marginalis nor the taenia medialis have yet established cartilaginous connexions with the planum supraseptale,

although the position of these future connexions is evident from the appearance of projections from the planum supraseptale itself. The hinder portion of the interorbital septum projects upwards and backwards towards the hinder part of the planum supraseptale, with which however it does not fuse.

In the nasal capsule the parietotectal cartilages are now more extensive, while the auditory capsules are now joined to one another above the brain by means of a slender bar of cartilage forming the tectum synoticum. This structure passes immediately behind the large and prominent endolymphatic sacs.

The crista sellaris is now a complete bar, separating the fenestra hypophyseos from the fenestra basicranialis. The distal end of the columella auris bears a process which extends forward in the tympanum, and which corresponds to the pars inferior of the hyostapes of Versluys's (1898) descriptions. At this stage, at least on one side of embryo D, this pars inferior is separate from the conical proximal portion of the columella auris, which confirms Versluys's statement (1908) that the proximal and distal ends of the columella auris have separate centres of chondrification.

The wall of the auditory capsule has now closed in round the footplate of the proximal end of the columella auris, with the result that the fenestra ovalis is reduced to its definitive size and that its aperture is blocked by the above-mentioned footplate. It is, however, important to remember that the columella auris arose as a separate cartilage without any connexion with the wall of the auditory capsule.

Stage 9 (embryo E, H.L. 5.25 mm., Text-figs. 17 and 18).—This is the last stage of which a complete description of the chondrocranium will be given, for it may be compared directly with the earlier of the stages described by Gaupp (1900). The side wall of the skull in the orbitotemporal region is now as complete as it will ever be. The taenia marginalis extends from the planum supraseptale to the roof of the auditory capsule; the pila metoptica joins the taenia medialis, which in turn is connected with the planum supraseptale in front, the pila accessoria above, and the pila antotica behind and beneath.

TEXT-FIGS. 17-20.

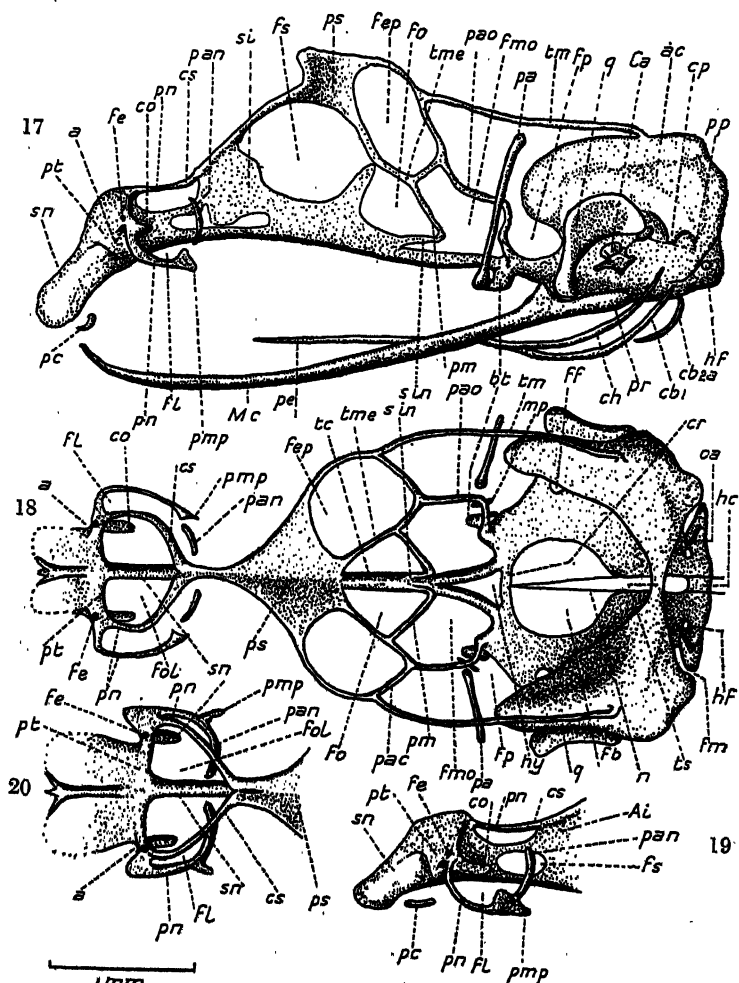


Fig. 17.—Lateral view from the left side of the skull.

Fig. 18.—Dorsal view of embryo E, stage 9, H.L. 5.25 mm.

Fig. 19.—Lateral view from the left side of the nasal capsule.

Fig. 20.—Dorsal view of embryo F, stage 10, H.L. 5.5 mm.

In this way the side wall of the skull shows four large openings on each side in this region: the fenestrae optica, epioptica,

metoptica, and prootica. The relations of the various nerves to these different apertures have been described by Gaupp (1900).

The basitrabecular processes are now large structures, and a feature of considerable interest is the appearance of a small independent piece of cartilage between the basitrabecular process and the base of the processus ascendens. This is the so-called meniscus pterygoideus of Howes and Swinnerton's (1901) description of *Sphenodon*, and the cartilago articularis ossis pterygoidei of Gaupp's (1900) description of *Lacerta*. A similar structure has been reported in *Emys* by Kunkel (1912). In his earlier work, Gaupp (1891) showed that the cartilago articularis was connected with the base of the processus ascendens in early stages, and subsequently (1902) he regarded it as the representative of the basal process of the palatoquadrate. This is probably correct. The processus ascendens is still an isolated cartilage, and it is to be noticed that the pterygoid process at its base has not yet developed (cf. stages described by Gaupp). In sections of an embryo slightly older than that here described it has been possible to confirm Broom's (1924) observations (on the lacertilians *Zonurus*, *Eremias*, and *Mabuia*) that the strand of dense tissue which connects the base of the processus ascendens with the quadrate (described by Gaupp, 1891) sometimes undergoes chondrification. The quadrate cartilage of *Lacerta* is therefore not always free at this stage.

As regards the columella auris, the pars inferior of the distal end is now fairly well developed, and the proximal and distal ends have joined to form a single rod, expanded at each extremity. Dorsal to the columella a small cartilage is seen lodged between the head of the quadrate and the crista parotica. Sections show that this little cartilage is in blastematous connexion with the columella auris, of which it represents the processus dorsalis, or intercalary, or processus paroticus. As already mentioned, this structure eventually becomes joined on to the crista parotica, as Gaupp (1900), Versluys (1908), and Goodrich (1916) described.

The fissura metotica is still open posterodorsally, for the

occipital arches have not yet fused on to the hind wall of the auditory capsules. Otherwise, the fissura metotica has assumed its definitive form and bears the usual relations to the foramen perilymphaticum of the auditory capsule (de Beer, 1929). The foramen perilymphaticum is merely an unchondrified portion of the hinder part of the floor of the auditory capsule.

Perhaps the most interesting features which this stage presents are connected with the nasal capsule. The parietotectal cartilages have extended right and left of the nasal septum, and by now a considerable portion of the roof and side walls of the capsule have been formed. Attached to the posterolateral corners of the parietotectal cartilages a new element has appeared on each side. This is the paranasal cartilage.

The paranasal cartilage is shaped like a crescent with the convex side turned forward, and the two horns pointing backward, one above and the other below. The upper horn is attached to the free anterior end of the sphenethmoid commissure, and, slightly in front of this, the paranasal cartilage is attached to the parietotectal cartilage. In this way a pair of apertures is enclosed. Each of these apertures, which are the fenestrae olfactoriae, is bounded medially by the nasal septum, posterolaterally by the planum supraseptale and the sphenethmoid commissure, anterolaterally by the paranasal cartilage, and anteriorly by the parietotectal cartilage.

Near the point of attachment of the paranasal cartilage to the parietotectal cartilage is the foramen epiphaniale, through which the lateral branch of the ethmoid nerve leaves the cavity of the nasal capsule, and the position of this foramen is of importance. Immediately beneath the foramen epiphaniale the paranasal cartilage forms as it were a duplication of the side wall of the capsule, for it is situated laterally to that hindmost portion of the side wall which is formed by the parietotectal cartilage. Between these two cartilaginous walls there is therefore a space, the cavum conchale, which ends blindly behind, but opens forward by the so-called aditus conchae, and lodges the lateral nasal gland. The whole structure, which is shaped somewhat like a cone with the point directed backward, is

known as the concha, and it projects into the cavity of the definitive nasal capsule. The lower horn of the paranasal cartilage projects freely backward as the processus maxillaris posterior.

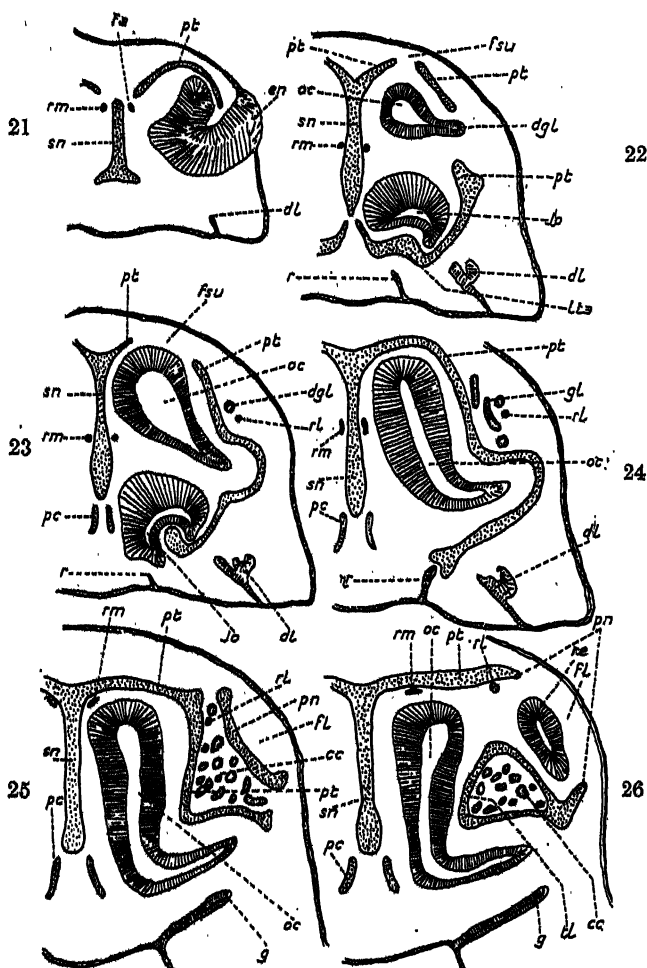
The hind wall of the nasal capsule is represented by an independent paired cartilage, the planum antorbitale, situated at the side of the nasal septum, between the root of the sphenethmoid commissure and the processus maxillaris posterior. The floor of the nasal capsule is represented only by the isolated rudiments of the paraseptal cartilages, which extend for only a short way beneath the ventral edge of the nasal septum.

Stage 10 (embryo F, H.L. 5.5 mm., Text-figs. 19 and 20).—The main portions of the skull of this stage show no appreciable advance over that of the previous one; only in the nasal capsule have important advances been made. There, the lower posteriorly directed horns of the paranasal cartilages have become attached to the planum antorbitale of their own side. The latter has, however, not yet established connexion with the sphenethmoid commissure. When that has happened, there will still be a large lateral opening in the hinder part of the side wall of the nasal capsule: the fenestra lateralis, through which it is possible to see the concha as it projects backward into the cavity of the nasal capsule. This condition is illustrated in figs. 8 and 13 of Gaupp's (1900) description of the skull of an embryo of *Lacerta* 31 mm. in length, and Born's (1879) fig. 1, Pl. VI, of an embryo of *Lacerta* ready to hatch.

Although Gaupp's descriptions of the nasal capsule are excellent, they are somewhat difficult to follow, and it is not easy to visualize the geometrical relations without a series of transverse sections. For this reason, a few sections through selected regions of the nasal capsule of an embryo of *Lacerta* slightly younger than the earliest of Gaupp's have been added (Text-figs. 21–8).

Text-fig. 21 passes through the external nostril and the fenestra narina through which the nasal sac communicates with the exterior. The roof of the capsule is formed by the front part of the parietotectal cartilage, and the section passes through the

TEXT-FIGS. 21-6.



Selected transverse sections. Series Jenkinson D.

Fig. 21, section 1-2-2; fig. 22, section 1-3-14; fig. 23, section 1-4-5; fig. 24, section 2-1-4; fig. 25, section 2-2-7; fig. 26, section 2-2-15.

small foramen apicale, through which the medial branch of the ethmoid nerve emerges.

Text-fig. 22 shows the hinder portion of the fenestra narina

through which the duct of the lateral nasal gland leaves the olfactory sac to run back laterally to the wall of the capsule. The roof of the capsule is here perforated by the fenestra superior, which appears to have no morphological significance.

The floor is formed by the lamina transversalis anterior, which is connected with the ventral edge of the parietotectal cartilage and was connected with the ventral edge of the nasal septum a few sections farther forward.

Text-fig. 23 is behind the lamina transversalis anterior, and Jacobson's organ is seen descending towards its opening into the buccal cavity. The fenestra superior is still shown, as are the paraseptal cartilages. Lateral to the true side wall of the capsule (formed by the parietotectal cartilage) may be seen the lateral branch of the ethmoid nerve and the duct of the lateral nasal gland.

Text-fig. 24 is behind the fenestra superior, and so the roof of the capsule is complete. The section passes through the anterior acini of the lateral gland, which are accommodated in a groove in the wall of the capsule. The lateral branch of the ethmoid nerve is still lateral to the side wall of the capsule.

Text-fig. 25 passes through the anterior region of the paranasal cartilage. It is lateral to the lateral nasal gland, which in turn is (together with the lateral branch of the ethmoid nerve) lateral to the true side wall. The gland therefore finds itself enclosed by cartilage in the cavum conchale, which the duct and the nerve have entered by its anterior opening: the *aditus conchae*.

In Text-fig. 26 the paranasal cartilage has become attached to the parietotectal cartilage, but it does not form a complete cartilaginous wall because of the large fenestra lateralis. The lateral branch of the ethmoid nerve is now median to the paranasal cartilage, and has entered the cavity of the nasal capsule (through its own small foramen epiphaniale). It is important to realize that the cavity of the concha (the *cavum conchale*) is really a part of extracapsular space, and it is lined throughout by what are really external capsular walls. The concha may be descriptively regarded as having been pushed into the cavity of the capsule at the spot where the paranasal cartilage becomes

attached to the parietotectal. Or, alternatively (and with a greater degree of probability), it may be said that the concha owes its existence to the fact that the paranasal cartilage has been reflexed forward, outside the side wall of the capsule

TEXT-FIGS. 27, 28.

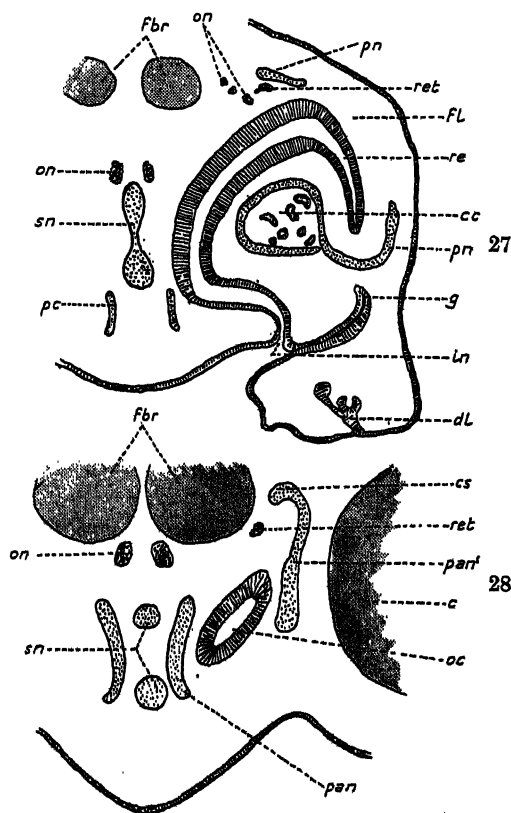


Fig. 27, section 2-4-4; fig. 28, section 2-5-8.

formed by the parietotectal cartilage. The result is that the cavity of the capsule bulges out and forward over the concha, and this bulge accommodates the recessus extraconchalis. The cause of this bulge is probably associated with the enormous size of the eyes, which press on the hind wall of the nasal

capsule, causing it to find the necessary accommodation for its contents in the manner described.

In Text-fig. 27 the recessus extraconchalis is still open to the side through the fenestra lateralis. The lateral and median branches of the ethmoid nerve are now united, dorsal to the branches of the olfactory nerve, forming the ramus ethmoidalis of the profundus branch of the trigeminal nerve.

In Text-fig. 28 the fenestra lateralis is no longer seen, for the planum antorbitale (itself attached to the lower horn of the paranasal cartilage) has established connexion with the sphenethmoid commissure (itself attached to the upper horn of the paranasal cartilage). The paraseptal cartilage has also run into the median portion of the planum antorbitale, and, a few sections farther back, the planum antorbitale will be seen forming a complete hind wall to the cavity of the nasal capsule.

III. DISCUSSION.

The only other lacertilian of which the earliest stages of development have been studied is *Ascalabotes*, by Sewertzoff (1900), and it must be noted that there are considerable differences between the conditions which he describes and those which are given in this paper. According to Sewertzoff, the trabeculae in *Ascalabotes* appear wide apart and separate from one another, whereas in *Lacerta* I have found them to be joined anteriorly to form a trabecula communis from their earliest appearance. Then Sewertzoff describes the appearance at an early stage of a crista sellaris, connected on each side with an 'alisphenoid' (meaning the pila antotica) and unconnected either with the trabeculae in front or the parachordals behind. In *Lacerta*, however, as described in this paper, the chondrification of the crista sellaris and pila antotica occurs comparatively late, and in continuity with the cartilages of the floor of the skull. In this respect, my observations are partly in agreement with Parker's (1879) on *Lacerta*. His earliest figured stage (Pl. XXXIX, fig. 1) shows the trabeculae attached to the parachordals; and diverging freely anteriorly. On the other hand, in his next stage (Pl. XXXIX, fig. 2) the trabeculae

have fused to form a trabecula communis but there is no crista sellaris.

The comparison between the chondrocranium of *Lacerta* and that of other reptiles so far known is reserved for a later section of this discussion. It may, however, be noted here that Shaner (1926), who studied early stages of development of *Chrysemys*, found conditions comparable to those described by Sewertzoff for *Ascalabotes*; the trabeculae were free anteriorly and a structure was present corresponding to the crista sellaris.

A discussion of the relations of the cartilages to the nerves and blood-vessels is unnecessary here, for they have been summarized in a previous work (de Beer, 1926).

A. The nasal capsule.

The nasal capsule of the lacertilian is a complicated structure, and a welcome light on its interpretation has been thrown by the recently acquired knowledge of the development of the nasal capsule in certain mammals. It has been shown by Terry (1917) in the cat, and confirmed by de Beer and Woodger in the rabbit, that three elements take part in the formation of the nasal capsule. There is (1) the parietotectal cartilage, which is continuous with the dorsal edge of the nasal septum, and forms the anterior part of the roof and side wall of the capsule; (2) the paranasal cartilage, which forms the hind part of the side wall; and (3) the planum antorbitale which forms the hind wall. In the mammals mentioned these elements chondrify independently of one another, and subsequently become connected. Where the paranasal cartilage joins the parietotectal, the foramen epiphaniale remains as a witness of the former space separating them. Further, as the paranasal cartilage overlaps the parietotectal, the posterior edge of the side wall formed by the latter projects into the cavity of the nasal capsule as the so-called crista semicircularis. Now, although in the lizard it has not been possible to find a stage at which the paranasal cartilage was separate from the parietotectal, yet the position of the foramen epiphaniale may be taken as an indication of the line

of demarcation between these two elements. Further, this line is also that of the *aditus conchae*. Allowance has of course to be made for the fact that the paranasal cartilage is widely fenestrated (by the *fenestra lateralis*) in *Lacerta*, but this is not the case in *Eumeces* (Rice, 1920) or in *Lygosoma* (Pearson, 1921), where this cartilage forms an unbroken wall. Now the median wall of the concha of the lacertilian (representing the posterior portion of the side wall of the capsule formed by the parietotectal cartilage) bears relations which are very comparable to those of the *crista semicircularis* of the mammal, and the mammalian condition would be still further approached if the cavity of the concha of the reptilian nasal capsule were obliterated by the approximation and fusion of its median and lateral walls, or if the lateral wall of the concha disappeared and the *aditus conchae* were closed by the paranasal cartilage.

It is not proposed to homologize the reptilian concha with the mammalian *crista semicircularis*, but an investigation into the causes contributory to the formation of the former might throw some light on the interpretation of the latter. Seydel (1896) considered the concha as an inpushing of the capsular wall due to the development of the lateral nasal gland. Gaupp (1900), however, inclined to the view that the accommodation of the gland in the concha is a passive result of another process, viz. the expansion of the cavity of the nasal capsule resulting in a bulging outward and forward of the side wall over the concha. It seems further not improbable that this process may have been associated with the huge size of the eye in the lacertilian, which presses on the capsule from behind.

However, the latter factor is probably less important in the case of the other reptiles which possess a concha: crocodiles and snakes; and it can hardly be appealed to in the case of the mammals.

The formation of the concha is therefore probably associated with an expansion of the cavity of the nasal capsule. The *recessus extraconchalis* of the lizard may be regarded as comparable to the mammalian *recessus anterior*; but the concha

would not correspond to the mammalian maxilloturbinal as Gaupp (1900) supposes.

Among other reptiles the presence of a concha is reported by Peyer (1912) for *Vipera*; by Brock (1929) for *Leptodeira*; and by Shiino (1914) for *Crocodylus*. In birds Tonkoff (1900) has shown for *Gallus* that a structure corresponding to the reptilian concha is present.

B. The fenestra ovalis and the basicapsular fenestra.

In many animals the cartilaginous auditory capsule becomes attached to the lateral edge of the parachordal by two commissures known respectively as the anterior and posterior basicapsular commissures (e. g. in the trout, de Beer, 1927). A gap is thus formed between the capsule laterally and the parachordal medially known as the fenestra basicapsularis, and which ultimately becomes obliterated as the chondrification of the floor of the auditory capsule is completed. Now, in several places in his treatise, Gaupp (1906) states that this basicapsular fenestra becomes the fenestra ovalis (=fenestra vestibuli) of the auditory capsule of tetrapods, while it becomes obliterated in the fish (cf. loc. cit., pp. 588, 725). As it stands, this statement is slightly misleading, for it would lead one to suppose that the medial border of the fenestra ovalis (into which the footplate of the columella auris fits) is formed of parachordal and therefore axial cartilage. There is no doubt that the medial border of the foramen ovale is formed of true capsular cartilage. However, as in many forms the chondrification of the floor of the capsule is delayed, the fenestra ovalis has for a time no medial border, and is therefore confluent with the basicapsular fenestra. It is therefore hardly legitimate to say that the fenestra ovalis is a remnant of the basicapsular fenestra itself.

When the condition in the tetrapod is compared with that of *Selachii*, it is clear that the basicapsular fenestra of the latter has no claim to represent any part of the fenestra ovalis. The position of the fenestra ovalis is morphologically indicated by the point of articulation of the hyomandibula, which is a

considerable distance lateral to the basicapsular fenestra. In this connexion it is of the greatest interest to note that van Wijhe (1924) has actually found a small fenestra ovalis in *Heptan-chus*, situated where one would expect it, viz. 'in the under part of the fossa for the hyomandibula'.

C. The planum supraseptale, ala orbitalis, and interorbital septum.

Much of the peculiarity of the skull of the lizard is due to the fact that the floor of the cranial cavity in the orbital region is lifted high above the level of the trabecula communis, a tall vertical interorbital septum being intercalated in between. It is clear that this modification is directly related to the large size of the eyes in these animals.

As regards the interorbital septum itself, the conditions in *Sphenodon* as reported by Schauinsland (1900) and by Howes and Swinnerton (1901) lead to the conclusion that it is really a distinct element, separate from the trabecula communis. Fuchs (1915) approaches the matter from a different point of view in his study of *Chelone*, for he denies that the trabecula communis extends as far forward as this. The conditions in *Lacerta* here described lend support to the view that the interorbital septum is an independent structure, which rapidly acquires connexion with the trabecula. At all events the matter seems to be of little importance. The membranous skull (*dura mater*) is stretched up from the trabecula (as the fore-brain is lifted dorsally) and gives rise to a vertical wall between the orbits, in which chondrification sets in.

The formation of the interorbital septum results in important modifications of the orbital cartilage. This structure no longer springs up from two pairs of roots direct from the trabecula. The posterior pair (the *pila metoptica*) is present, but the anterior pair has been lost. Further, the orbital cartilages in their anterior portion have been pressed together between the orbits so that their medial borders meet in the middle line, forming the planum supraseptale. This explains why the sphenethmoid commissures leave the planum supraseptale near

the middle line, and diverge as they run forward. It may be noted that an interorbital septum is present in certain mammals (Primates, Rodents), and in these the orbital cartilage (ala orbitalis) springing from the dorsal edge of the septum approximates to the form of a planum suprasetale.

The posterior portion of the orbital cartilage is in a reduced condition, being represented only by the taenia medialis, taenia marginalis, pila accessoria, pila metoptica, and pila antotica, which form a very slender framework. This region of the skull presents features of interest for comparison with other reptiles, from which interesting conclusions as to the affinities of the lacertilians may be drawn.

D. The phylogenetic position of the Lacertilia in the light of the chondrocranium.

The affinities of the Lacertilia have been a matter of controversy for some time. Long ago Huxley (1871) considered that the Lacertilia had lost the lower temporal bar which is still preserved in *Sphenodon*. Whether they are regarded, as Huxley would have and as Broom (1924) does, as members of the Diapsida in which the lower temporal fossa has been lost, or, with Williston (1925), as Parapsida which have never possessed a lower temporal fossa, the fact remains that these distinctions are based primarily on the configurations of the temporal arches formed by the dermal bones in the osteocranium. It becomes of interest to inquire whether any independent indications of affinity are given by the chondrocrania of Lacertilia and other reptiles. An attempt at a comparison of this kind is now possible, since investigations have been made by modern methods into the structure of the chondrocranium of all the principal groups of surviving reptiles.

It must be said at once that the snakes must be excluded from any such comparison, for their skulls are so peculiar and specialized as to supply little material for profitable comparison. As Brock (1929) has pointed out, the evidence in favour of the lacertilian origin of the Ophidia is debatable. There remains, then, *Sphenodon*, the Crocodilia, and the Chelonia,

with which the *Lacertilia* may be compared. In all of these there is the same general form of a tropitrahic skull with a trabecula communis, an interorbital septum, and a planum supraseptale, and in all the posterior portion of the orbital cartilage is more or less reduced. This reduction of the orbital cartilage follows definite lines, and it is interesting to note that the condition in the *Lacertilia* (*Lacerta*, Gaupp, 1900; *Eumeces*, Rice, 1920) is identical in plan with that found in *Crocodylia* (Shiino, 1914) and in *Sphenodon* (Schauinsland, 1900; Howes and Swinnerton, 1901). In each case it is easy to identify the four fenestrae: optica, metoptica, epioptica, and prootica; separated from one another by the various taeniae and pilae already described. These cartilaginous struts are more slender in the *Lacertilia* than in the others, indicating a greater degree of specialization. In the *Chelonia* the conditions are more variable. In *Dermochelys* (Nick, 1912) the side wall of the skull in this region is fairly substantial, while in *Chelone* (Fuchs, 1915) it has reached approximately the same degree of reduction as in the crocodile. *Emys* (Kunkel, 1912), *Chrysemys* (Shaner, 1926), and *Chelydra* (Nick, 1912) show still greater reduction, but the same general plan of structure can be recognized. The fact that the lacertilians conform to this plan suggests that their affinities lie with these animals.

This conclusion is strengthened by a consideration of two other regions of the skull. The concha of the nasal capsule, present in lacertilians, is also present in a precisely comparable condition in the crocodile (Shiino, 1914). (The concha is present in the snakes: *Vipera*, Peyer, 1912; *Leptodeira*, Brock, 1929.) The pterygoquadrate presents great similarities in *Sphenodon*, *Crocodylus*, and *Chelonia*, consisting of an otic process connected to the base of an ascending process, from which a pterygoid process projects forward. In the *Lacertilia* all these elements are present in precisely comparable conditions, although the connexion between the bases of the ascending and otic processes is slender and transient.

As far as the evidence from the chondrocranium goes, it

shows that the Lacertilia have several points of similarity with *Sphenodon* and the crocodiles, that is to say, with Diapsida, and it supports Broom's views concerning the derivation of the Lacertilia from this group.

IV. SUMMARY.

1. The embryology of the chondrocranium has been studied in ten stages of the development of *Lacerta agilis*.
2. The splanchnocranium chondrifies before the neurocranium.
3. The crista sellaris has a paired and belated origin.
4. The constituents of the nasal capsule of *Lacerta* agree generally with those of mammals.
5. It is pointed out that the foramen ovale is not a derivative of the fenestra basicapsularis.
6. The otic process of *Lacerta* has a temporary cartilaginous connexion with the processus ascendens.
7. Points of similarity between the chondrocrania of Lacertilia and of other reptiles lead to the conclusion that the Lacertilia are derived from Diapsid reptiles.

V. LIST OF LITERATURE CITED.

- Born, G. (1879).—"Die Nasenhöhlen und der Thränennasengang der amnioten Wirbelthiere", 'Morph. Jahrb.', 5.
- Brook, G. T. (1929).—"On the development of the skull of *Leptodeira hotamboia*", 'Quart. Journ. Micr. Sci.', 73.
- Broom, R. (1924).—"On the classification of the Reptiles", 'Bull. Amer. Mus. Nat. Hist.', 51.
- de Beer, G. R. (1926).—"Studies on the vertebrate head. II. The orbito-temporal region of the skull", 'Quart. Journ. Micr. Sci.', 70.
- (1927).—"The early development of the chondrocranium of *Salmo fario*", *ibid.*, 71.
- (1929).—"The development of the skull of the shrew", 'Phil. Trans. Roy. Soc.', B, 217.
- de Beer, G. R., and Woodger, J. H.—(In the press) 'The early development of the skull of the rabbit.'
- Fuchs, H. (1915).—"Über den Bau und die Entwicklung des Schädels der *Chelone imbricata*", 'Voeltzkow: Reise in Ostafrika'.
- Gaupp, E. (1891).—"Die Columella der Kionokranen Saurier", 'Anat. Anz.', 6.

- Gaupp, E. (1900).—"Das Chondrocranium von *Lacerta agilis*", 'Anat. Hefte', 15.
- (1902).—"Über die Ala temporalis des Säugerschädels und die Regio orbitalis einiger anderen Wirbeltierschädel", *ibid.*, 19.
- (1906).—"Die Entwicklung des Kopfskelettes", Hertwig's 'Handbuch d. verg. u. exp. Entwickl. d. Wirb.', 3.
- Goodrich, E. S. (1911).—"On the segmentation of the occipital region of the head in the Batrachia Urodela", 'Proc. Zool. Soc.'
- (1916).—"The Chordatympani and middle ear in Reptiles, Birds, and Mammals", 'Quart. Journ. Micr. Sci.', 61.
- (1918).—"On the development of the segments of the head in Scyllium", *ibid.*, 63.
- Howes, G. B., and Swinnerton, H. H. (1901).—"On the development of the skeleton of the tuatara", 'Trans. Zool. Soc.', 16.
- Huxley, T. H. (1871).—"A manual of the Anatomy of Vertebrated Animals."
- Kunkel, B. W. (1912).—"The development of the skull of *Emys*", 'Journ. Morph.', 23.
- Leydig, F. (1872).—"Die in Deutschland lebenden Arten der Säurier."
- Nick, L. (1912).—"Das Kopfskelett von *Dermochelys coriacea*", 'Zool. Jahrb. Abt. f. Anat.', 33.
- Parker, W. K. (1879).—"On the structure and development of the skull in the Lacertilia", 'Phil. Trans. Roy. Soc.', 170.
- Pearson, H. S. (1921).—"The skull and some related structures of a late embryo of *Lygosoma*", 'Journ. Anat.', 56.
- Peyer, B. (1912).—"Die Entwicklung des Schädel skelettes von *Vipera aspis*", 'Morph. Jahrb.', 44.
- Rice, E. L. (1920).—"The development of the skull in the Skink, *Eumeces quinquelineatus*", 'Journ. Morph.', 34.
- Schauinsland, H. (1900).—"Weitere Beiträge zur Entwicklungsgeschichte der Hatteria", 'Arch. f. mikr. Anat.', 56. (Also: 1903.—'Zoologica.')
- Sewertzoff, A. N. (1897).—"Beitrag zur Entwicklungsgeschichte des Wirbeltierschädels", 'Anat. Anz.', 13.
- (1900).—"Zur Entwicklungsgeschichte von *Ascalabotes fasciularis*", *ibid.*, 18.
- Seydel, O. (1896).—"Über die Nasenhöhle und das Jacobson'sche Organ der Land- und Sumpfschildkröten", 'Festschr. f. Gegenbaur', 2.
- Shaner, R. F. (1926).—"The development of the skull of the turtle, with remarks on fossil reptile skulls", 'Anat. Rec.', 32.
- Shiino, K. (1914).—"Das Chondrocranium von *Crocodylus* mit Berücksichtigung der Gehirnnerven und Kopfgefäße", 'Anat. Hefte', 50.
- Terry, R. J. (1917).—"The primordial cranium of the cat", 'Journ. Morph.', 29.
- Tonkoff, W. (1900).—"Zur Entwicklungsgeschichte des Hühnerschädels", 'Anat. Anz.', 18.

- van Wijhe, J. W. (1902).—"A new method for demonstrating cartilaginous mikroskeletons", 'Kon. Akad. v. Wetensc. t. Amsterdam.'
- (1922).—"Frühe Entwicklungsstadien des Kopf- und Rumpfskeletts von *Acanthias vulgaris*", 'Bijdr. t. d. Dierk.', 22.
- (1924).—"Thymus, spiracular sense organ and fenestra vestibuli (ovalis) in a 63 mm. long embryo of *Heptanchus einereus*", 'Kon. Akad. v. Wetensc. t. Amsterdam. Proc.', 26.
- Versluys, J. (1898).—"Die mittlere und äussere Ohrsphäre der Lacertilier und Rhynchocephalier", 'Zool. Jahrb. Abt. f. Anat.', 12.
- (1903).—"Entwicklung der Columella auris bei den Lacertiliern", *ibid.*, 19.
- Williston, S. W. (1925).—"The osteology of the Reptiles." Harvard.

INDEX TO VOL. 73

NEW SERIES

Actinomyxidæ, chromosome cycle, Naville, 547.

Bacterial cell-mass in Weevil, Mansour, 421.

Beer, G. R. de. The Early Development of the Chondrocranium of the Lizard, 708-739.

Berkeley, C. The Green Bodies of the Intestinal Wall of certain Chaetopteridæ, 465-476, pl. 26.

Bowen, R. H. See Frew, 197.

Brook, G. T. On the Development of the Skull of *Leptodeira hotamboia*, 289-334, pls. 12, 13.

— The Formation and Fate of the Operculum and gill-chambers in the tadpole of *Rana temporaria*, 335-344.

Brown, V. E. Cytology and Binary Fission of *Peranema*, 403-420, pls. 19-21.

Cats, thyroid glands, Lowe, 577.

Chaetopteridæ, green, bodies in intestinal wall, Berkeley, 465.

Chromosome cycle in Actinomyxidæ, Naville, 547.

Ciliata; Chilodon, conjugation of triploid, MacDougall, 215.

— *Physophaga sappheira*, Percy, 107.

Conjugation of triploid Chilodon, MacDougall; 215.

Conus arteriosus in fish, Parsons, 145.

Cucumaria gregarines, Pixell Goodrich, 275.

Cytology; radium on root-tips Patton, 633.

Dawes, B. The Histology of the Alimentary Tract of the Plaice (*Pleuronectes platessa*), 243-274.

Dermaptera; development of genitalia, Nel, 25.

Development; skull *Leptodeira*, Brook, 289.

— Operculum and gill-chambers of tadpole, Brook, 335.

— Mesonephros of *Rana*, Gray, 507.

— Chondrocranium of lizard, de Beer, 708.

Elasmobranchs; Lipin secretion in Interrenal, Fraser, 121.

Enteropneusta, metamerism, van der Horst, 393.

Kaulner, G. H. The Early Phases of the First Oocyte Division as seen in Life, in *Ophelia geniculata*, 225-242.

Fish, Conus arteriosus, Parsons, 145.

Fraser, A. E. H. Lipin Secretion in the Elasmobranch Interrenal, 121-134, pl. 7, 8.

Frew, P., and Bowen, R. H. Nuclear behaviour in the Mitosis of Plant-cells, 197-214, pl. 11.

